1976

# Autonomic Regulation Of Calcium And Amylase Secretion By Rat Salivary Glands.

Choogiart Sucanthapree
*University of Alabama at Birmingham*

## INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

University Microfilms International
300 North Zeeb Road
Ann Arbor, Michigan 48106 USA
St. John's Road, Tyler's Green
High Wycombe, Bucks, England HP10 8HR

# AUTONOMIC REGULATION OF CALCIUM AND AMYLASE SECRETION BY RAT SALIVARY GLANDS

by

CHOOGIART SUCANTHAPREE

A DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the
Department of Physiology and Biophysics in
The Graduate School,
University of Alabama in Birmingham

BIRMINGHAM, ALABAMA

1976

ACKNOWLEDGMENTS

I am indebted to Chulalongkorn University, Bangkok, Thailand for providing financial support throughout the course of my education.

My deepest gratitude is expressed to Dr. Leon H. Schneyer and Dr. Charlotte A. Schneyer for spending their invaluable time in aiding me during my research. Without their guidance, advice and encouragement, this degree would not have become a reality.

Collectively, I would like to thank Dr. Charlotte A. Schneyer, Dr. Geraldine Emerson, Dr. Richard Shoemaker, and Dr. Gerald L. Carlson for serving as members of my graduate committee. A special acknowledgment is extended to Dr. Leon H. Schneyer for serving as my graduate committee chairman.

I would also like to express my appreciation to Mr. Herman Forrest for his taking care of my experimental animals.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| A.T. = | auriculotemporal nerve |
| CHORDA = | chorda tympani nerve |
| cyclic AMP = | cyclic adenosine 3',5'-monophosphate |
| cyclic GMP = | cyclic guadenosine 3',5'-monophosphate |
| ISO. = | isoproterenol |
| PILO. = | pilocarpine |
| SYM. = | sympathetic nerve |

# INTRODUCTION

## Review of Literature

Salivary glands develop as chordal outgrowths from the oral epithelium. During the course of this development, the epithelial outgrowths assume a tubular form and ultimately become the branching duct system of the mature gland. The acinar structures, which form the proximal, blind ends of the mature ductal tree develop later, often postnatally, as outgrowths of the terminal tubules. Thus, the mature gland is racemose in structure. Acini comprise the most proximal element. The acinar lumen is contiguous with the lumen of the first proximal element of the mature duct system, the intercalated duct. Intercalated ducts converge to form striated ducts which further converge to form excretory ducts. In some glands, notably rodent submaxillary, the proximal portion of the striated duct is further distinguished by the presence of intracellular granules and is separately identified as the granular duct, or tubule. Finally, by further convergence, excretory ducts join to form the main excretory duct which, usually singly, terminates in the oral cavity (40, 42).

In salivary glands, ducts as well as acini have secretory functions. Historically, secretory functions have been associated

with acini for a longer time. However, in recent years it has become clear that the duct system of salivary glands serves as more than a simple conduit for conducting secretions to the mouth, and that important secretory events occur at ductal loci (24, 54). Functions of the two sets of structures, as currently conceived, can with moderate simplification be summarized as follows. Acini elaborate the first or "precursor" secretion. This fluid contains virtually all of the protein which is ultimately secreted, and, similarly, nearly all of the water. The osmotically important ions, sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) are present in approximately plasma-like concentrations and, consistent with this, acinar fluid (or acinar, intercalated-duct fluid) is essentially isotonic. In the striated and excretory ducts, re-equilibration of the major electrolytes occurs. Sodium and chloride are reabsorbed, and potassium and bicarbonate are secreted, but to lesser extent. Water shows little net movement across the ductal wall. As a consequence, final saliva generally shows potassium concentrations which exceed those of serum while sodium concentration is often appreciably lower than in serum. Osmolality is frequently low and saliva is usually hypotonic (24, 38, 42, 54).

The principal protein in saliva from many glands is the digestive enzyme, amylase. This enzyme is at a remarkably high level in rat parotid gland, for example, although it is virtually absent from rat submaxillary (41, 42). The enzyme is formed at the microsomes on the endoplasmic reticulum (15). Within the

cisternae, the enzyme molecules drift in an apical direction toward the Golgi complex, where they become localized in vacuoles. The vacuoles migrate toward the cell apex and after some condensation of their contents become recognizable as zymogen granules (6, 43, 45). The zymogen granules are discharged across the luminal membrane when the acinar cells are stimulated. Stimulation, in fact, leads to glandular depletion of amylase, which is later slowly resynthesized to again elevate glandular stores of the enzyme, a sequence which is generally referred to as a "secretory cycle."

In most salivary glands, including submaxillary and parotid glands of rat, secretion does not occur spontaneously, nor is secretion controlled by circulating hormones. Control of salivary secretion is exerted by the autonomic nervous innervation and mediated by the neurotransmitters, acetylcholine (parasympathetic) and norepinephrine (sympathetic) (25, 35, 39, 40, 42). In general, mammalian salivary glands all receive a parasympathetic cholinergic innervation, and this contains secretomotor and vasomotor fibers. Most glands are innervated by sympathetic adrenergic fibers as well, but only in some does this innervation contain secretomotor elements. Rat parotid and submaxillary glands receive secretomotor fibers from the sympathetic as well as the parasympathetic autonomic systems (4, 16, 17, 25, 28, 29, 49, 50). These salivary glands, like many others, respond to parasympathetic cholinergic stimulation by secretion of copious hypotonic secretion which is low in protein content (23, 32, 35, 36). The sodium concentration of this secretion,

especially in the case of the parotid gland, depends upon the degree of stimulation (35). Salivary [Na] is small at low intensities of stimulation and rises to reach a virtual plateau as stimulation intensity, and flow rate, are increased toward a maximum (35). This is not due to any increase in [Na] of the precursor fluid when stimulation and flow are increased. The effect (known as Heidenhain's Law) evidently occurs because the transport maximum (TM) of the duct cells for sodium is exceeded at high rates of delivery of the ion to the cells, and because of the small contact-time between cells and luminal fluid in the ducts. Salivary [K] on the other hand is less likely to change with changes in flow rate (35).

With sympathetic stimulation, flow rate is generally low, for example from rat parotid (36) or submaxillary gland (53), but the secretory fluid is high in protein concentration (23, 32, 36). The concentrations of potassium and bicarbonate are also characteristically high (32, 39, 53). The same effect can be obtained with adrenergic drugs (36, 53), and is especially marked with those agents which stimulate β-adrenergic receptors (32).

An unusually high concentration or output of potassium in saliva can also be noted with cholinergic or α-adrenergic stimulation during the first few minutes after the start of salivary flow. This "transient elevation" is considered to be the result of a release of glandular stores of potassium, due to a transient increase in K-permeability of the luminal and contraluminal

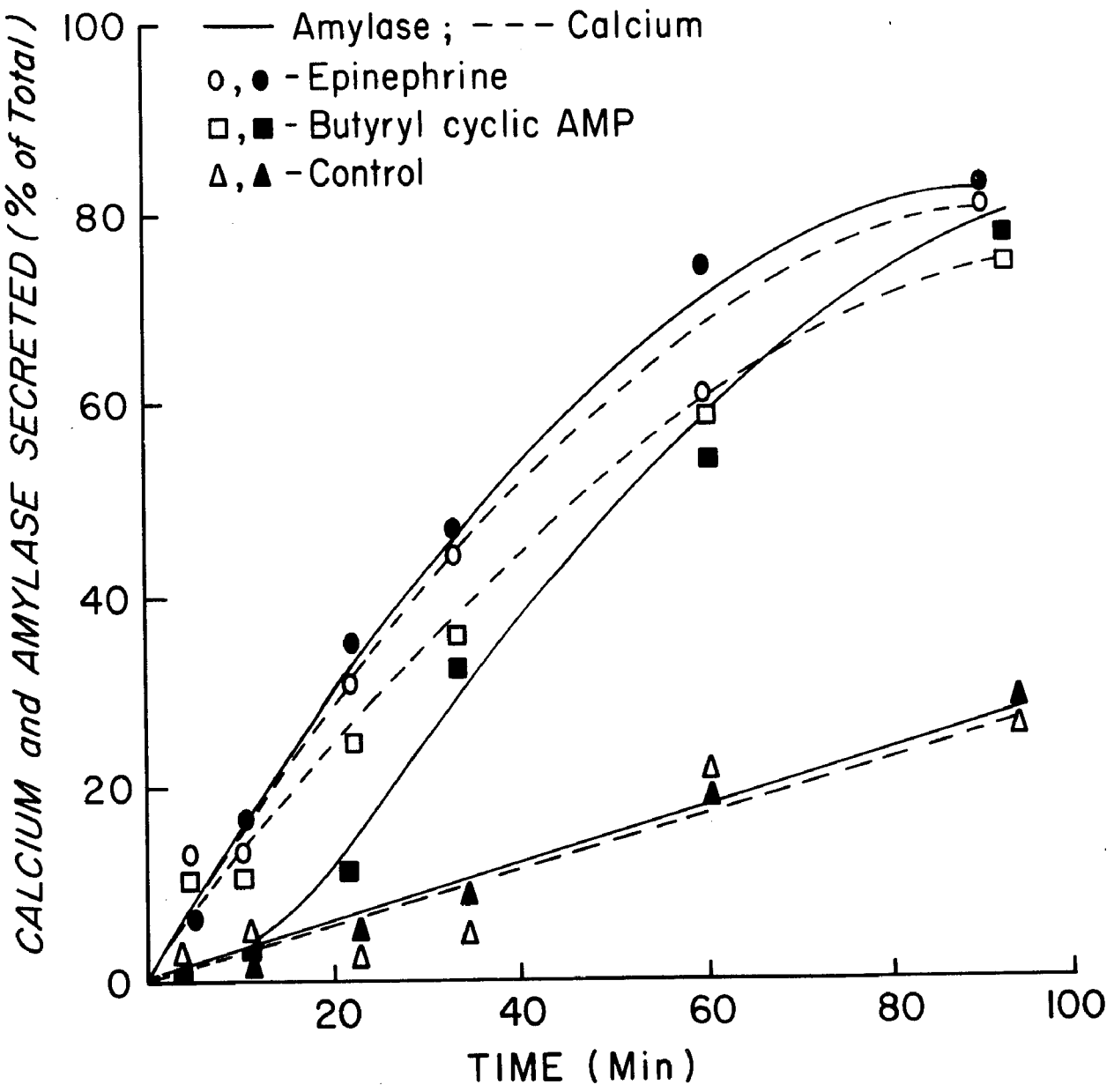
membranes, particularly of the acinar cells. The effect subsides after several minutes, even if stimulation is continued, and is followed by glandular reaccumulation of potassium, i.e., by the "negative transient" (5).

In rat submaxillary and parotid, gland tissue and secretion have been reported to contain strikingly high concentrations of calcium (15 to 25 mEq per kilogram wet weight of gland or per liter of secretion) (3, 7, 8, 11, 12). This calcium, which is evidently mainly complexed to organic macromolecules, is primarily localized in acinar cells (13, 51). Calcium is now known to play an important role in coupling the secretory response of salivary gland to the stimulus (14, 30, 31). Secretion of water and of protein are particularly affected by a decrease in extracellular calcium. With rat parotid gland, it has been shown that transmission of a message from the adrenergic receptor site probably involves both cyclic adenosine 3',5'-monophosphate (cyclic AMP) and $Ca^{2+}$, at least when β-adrenergic receptors are involved (2, 26, 47, 52). Secretion of amylase by rat parotid gland has been shown clearly to be dependent on the availability of $Ca^{2+}$ to the secretory cells (30, 47). In addition, in rat parotid gland a further relationship has been suggested between calcium and the secretion of amylase (52). The relationship involves a definite stoichiometry between amylase and calcium present in secreted saliva, at least with tissue slices in vitro. The relationship has been observed when sympathomimetic agents are added

to incubated parotid slices and is reproduced as Figure 1. Presumably, calcium is packaged along with amylase at the Golgi organ (48).

This finding has several implications. Among these is the possibility that secretion of calcium, like secretion of amylase, can be used as an indicator of the type of autonomic stimulation. So far, it is amylase secretion mainly, and potassium secretion to some extent, which can be used to characterize the type of autonomic receptor which is involved in evoking secretion. These functional parameters of the secretory response to parasympathetic or sympathetic stimulation, or to the stimulatory actions of parasympathomimetic or sympathomimetic drugs, have been of great value in analyzing autonomic pathways which mediate responses to "normal" stimuli (as in feeding) (33) or the receptors which mediate the secretagogic action of various drugs which have unexpected side actions, such as the primarily parasympathomimetic agent, pilocarpine (35, 36), or the antitumor agent cyclocytidine (34). Agents which act through activation of β-adrenergic receptors, for example, invariably evoke parotid secretion which is very high in amylase and potassium. Thus, [K] may reach 60 mEq/l in adrenergically evoked rat parotid saliva (32, 36). It would be important to determine whether calcium levels of saliva, or gland, also could be used to indicate the type of autonomic receptor which mediates a particular secretory response. Investigations of this point formed the basis for this research.

Figure 1. Secretion of amylase and calcium from rat parotid gland slices by various modes of stimulation (52).


Amylase ; - - - Calcium
o , ● - Epinephrine
□ , ■ - Butyryl cyclic AMP
Δ , ▲ - Control
CALCIUM and AMYLASE SECRETED (% of Total)
100
80
60
40
20
0
20
40
60
80
100
TIME (Min)

Proposal

The presence of an unusually high concentration of calcium (approximately 15-20 mEq/kg wet weight) in rat salivary tissue, compared with other soft tissues (e.g., kidney and liver), was first reported by Dreisbach (7, 11). In the same work, Dreisbach found that [Ca] in rat saliva, at least from the submaxillary-sublingual gland complex, also was strikingly high (up to 20 mEq/1), especially when secretion was evoked by isoproterenol (11, 12). From subsequent reports on calcium in rat submaxillary gland and saliva, it became clear that most of the calcium is bound (9, 12). However, no estimates were made concerning the ionic activity of calcium in cell water or saliva.

Dreisbach (7, 11, 12) determined the course of calcium secretion by rat submaxillary gland when pilocarpine or isoproterenol was used as the stimulating agent. With pilocarpine, secretion of calcium in saliva was found to occur at high, but progressively decreasing concentration over the first forty minutes of stimulation, and then to plateau, at a level near 1 mEq/1. Balance studies showed that the calcium secreted during the first forty minutes of stimulation, over and above that which would have been secreted under steady state conditions, closely matched the decrease in calcium which occurred in the gland during the same period. Evidently, a transient elevation occurs in the course of calcium secretion, as it does with potassium, and this calcium transient is unusually long lasting.

Consistent with this, it was found by Dreisbach (10) that the transfer of calcium into or out of the gland, in vitro, in incubated tissue, is enhanced by acetylcholine or by epinephrine.

In later work, Dreisbach (11, 12) showed that the β-adrenergic agonist, isoproterenol, induces even greater depletion of calcium from rat submaxillary gland than occurs with pilocarpine. In the saliva evoked by isoproterenol injection, the transient elevation of calcium is longer lasting (over 1 hr) than in pilocarpine-evoked saliva, but the plateau values (steady-state secretion) of calcium reached thereafter are similar (near 1 mEq/l) with the two drugs (7, 11). Protein concentration, but not concentration of sialic acid, was found to be directly proportional to calcium concentration of rat submaxillary saliva, evoked by isoproterenol or pilocarpine (12). Essentially all of the calcium in this saliva was found to be dialyzable (12). The cellular location of the salivary gland calcium was found by radioautography to be mainly acinar (13, 51).

Two other reports must because of their bearing on this research be considered. These are from Michael Schramm's research group at Hebrew University. In the first report, Selinger, Naim and Lasser (48) discussed the uptake and possible intracellular distribution of calcium by rat parotid and submaxillary glands. Calcium was found to be accumulated in these glands by the action of a non-mitochondrial calcium pump. The findings were made by using parotid gland homogenate, and although the actual identity

of the cellular structure associated with the pump could not be definitely established, the fraction in which calcium was accumulated to the greatest degree was the microsomal fraction. Electron microscopic examination indicated that smooth membrane vesicles predominated in the floating portion from this fraction. It was suggested that it is mainly in the Golgi apparatus that calcium is accumulated (48).

In the second report, Wallach and Schramm (52) described a parallel, but not linear, secretion of amylase and calcium from rat parotid slices when epinephrine or dibutyryl cyclic AMP was used as a stimulating agent. Subcellular fractionation of the gland showed that the highest concentrations of amylase and calcium were in the secretory granule fraction. Moreover, the ratio, nanomoles calcium/mg protein, of the secretory granule fraction was similar to that of the secreted material. It was concluded that calcium is packaged and secreted with exportable protein in the rat parotid gland.

Thus, the earlier work of Dreisbach and of the Schramm group suggest a parallelism in accumulation and secretion of calcium and protein by rat salivary glands. This relationship has been further investigated and tested in the present research. The approach has been to use rat parotid and submaxillary glands, in vivo, as the secretory system of choice. Moreover, the emphasis has been on a comparison of amylase and calcium secretion in

relation to the mode of stimulation, using modes which include direct stimulation of the autonomic innervation, as well as administration of secretagogue drugs. Stimulation of the autonomic nerves which innervate the gland has been used, for the first time in studying the calcium-amylase relationship in the saliva. The relationship between secretion of calcium and of amylase has been considered also in relation to duration of stimulation. Both parotid saliva (probably for the first time, since Dreisbach erroneously used lacrimal instead of parotid gland) and gland have been examined. The study started with an examination of the kinetics of calcium secretion by rat submaxillary gland, so that the early findings of Dreisbach could be reexamined, and then enlarged to examination of the calcium-amylase relationship in secretion by rat parotid gland, *in vivo*.

# MATERIALS AND METHODS

## Experimental animals

Male Long-Evans rats, 4 to 6 months old, and weighing between 300 to 400 grams were used in these experiments. They were maintained on Purina lab chow and water ad libitum until 18 hours before experimentation when food but not water was removed. The rats were anesthetized by intraperitoneal administration of sodium pentobarbital in doses of 50 milligrams per kilogram of body weight. Following the anesthetization, the trachea was isolated by midline incision and cannulated by polyethylene tubing, to avoid the possibility of respiratory complications.

## Collection of saliva samples

For collection of submaxillary saliva, a fine polyethylene cannula (Clay-Adams PE 10) was inserted to a distance of about 4 millimeters into the oral opening of one submaxillary duct (53). The saliva was collected from the end of the cannula, using Corning disposable micropipettes of 10 to 100 µl volume. Parotid saliva was collected by micropipette directly from the cut end of the gland duct (36). Sample volumes of 10 to 100 µl were used, undiluted, for determination of salivary calcium concentration. Volumes of 1 µl

of parotid saliva were used for amylase analysis, after dilution, 1:10,000, in phosphate-buffered (pH 7) sodium chloride solution (0.05 M). Samples for amylase analysis were refrigerated (4°C) until used, but not more than two days.

Preparation of glands for analysis

Parotid and submaxillary glands were rapidly removed from the anesthetized animal and weighed on a torsion balance. The parotid glands were then minced, and the mince was divided into two aliquots, each of which was individually weighed. One aliquot, weighing between 90 and 120 milligrams, was placed in a small vessel to which a few drops of normal saline were added, for subsequent determination of amylase activity. The remaining tissue aliquot was placed in a crucible for subsequent ashing and determination of calcium concentration. The portion of the gland for amylase was kept frozen at -15°C (for not longer than two weeks) until analyzed. The tissue sample to be ashed was placed in a Vycor crucible and dried at 105°C overnight. The dry tissue was then placed in a desiccator for two days, weighed for determination of water content, and then dry-ashed for calcium determination. With submaxillary, the entire gland was used for calcium determination. With either submaxillary or parotid, when stimulated glands were removed, this was done as quickly as possible after the end of a period of stimulation. Unstimulated glands from control rats or the unstimulated contralateral glands of experimental animals served as controls.

Stimulation of gland to secrete saliva

Saliva was elicited by administration of autonomic drugs or by stimulation of neural pathways. The β-adrenergic agent, isoproterenol hydrochloride (Winthrop Laboratories, New York) and the parasympathetic agent, pilocarpine hydrochloride (Mallinckrodt Chemical Works) were the autonomic drugs administered. Isoproterenol solution was prepared fresh at the time of experimentation and administered intraperitoneally in doses of 8 mg/rat. Pilocarpine was kept refrigerated in solution and administered intraperitoneally in doses of 5 mg/kg body weight.

With stimulation of neural pathways, a Grass Stimulator was used to deliver stimuli of supramaximal intensity. The instrument was set to deliver square wave pulses of 4 volts at a frequency of 20 pulses per second and a duration of 5 milliseconds (39, 53). The output of the stimulator was connected directly to bipolar electrodes. For parasympathetic nerve stimulation of submaxillary gland, the chorda tympani was stimulated as it coursed in the main duct (53). The submaxillary and sublingual ducts were isolated from the surrounding tissue about one centimeter. The bipolar electrodes were applied to the main submaxillary duct at a point close to the gland hilus. A thin strip of Parafilm was inserted under the electrodes to reduce current spread during stimulation. The sympathetic fibers were stimulated at the common carotid artery. The carotid sheath was isolated and the vagus nerve was separated

from the common carotid artery. The bipolar electrodes were placed around the carotid artery just below its bifurcation (39). In some cases, the sympathetic fibers were stimulated at the superior cervical ganglion itself. Spread of current from the electrodes was prevented by insertion of a Parafilm sheath under the electrodes. The parasympathetic activity of the parotid gland was obtained by electrical stimulation of the auriculotemporal nerve. The nerve was approached by separating the masseter and temporal muscles and cleared so that bipolar electrodes could be put in place (35).

Determination of calcium concentrations

Calcium concentrations of salivary samples were determined by automatic calcium titrator (Fiske Associates, Inc.). This instrument has successfully been used for determination of calcium in serum (1). The instrument incorporates a motorized buret, a fluorometer, and a digital readout that is responsive to quenching of the fluorescent calcium-calcein complex by the chelating agent, ethyleneglycol tetracetic acid. In these experiments a calcium standard was analyzed before and after each group of ten salivary samples. The average of the readings from the standard was used as the reference in calculating concentrations of calcium in the saliva and tissue samples.

The calcium level in glands was determined using the dried tissue. Glands were ashed overnight in an oven at 540°C. After cooling, the ash was dissolved in 0.5 ml of 1 N HCl and later

was neutralized with 0.5 ml of 1 N KOH. Aliquots of 50 to 100 µl were used for titration of calcium. The method of calcium analysis used here was tested by comparison of values from paired aliquots analyzed by titration and by atomic absorption spectroscopy, respectively. Agreement between values obtained by the two methods was within 3% (2.6 ± 2.6%, mean difference ± standard error of the mean). With the titration method, moreover, recoveries of calcium, when calcium was added to submaxillary gland, averaged 100.3%, with a standard error of 0.5%. The tests showed that this method was reliable in determining calcium level of salivary glands.

Determination of amylase activities

Amylase activities of saliva samples were determined by the method of Myers et al. (27), modified for use as a micromethod (21). Minor modifications in this determination were made. The 0.5 ml of diluted saliva was incubated at 37°C in 2 ml of soluble starch, 4 mg/ml in buffered saline, for exactly 15 minutes. The reaction was stopped by saturating the reaction mixture with dry picric acid. The precipitate was filtered. One milliliter of 15% sodium carbonate was added to 1 ml of filtrate, in a Klett tube. The tube was placed in a boiling water bath for 20 minutes. The solution was diluted to 10 ml and read in a Klett photoelectric colorimeter with a filter, No. 54, green, having a transmittance of 520 to 580 mµ. The instrument was set at zero with the picric acid blank. Amylase activity was expressed as milligrams of reducing substance, as

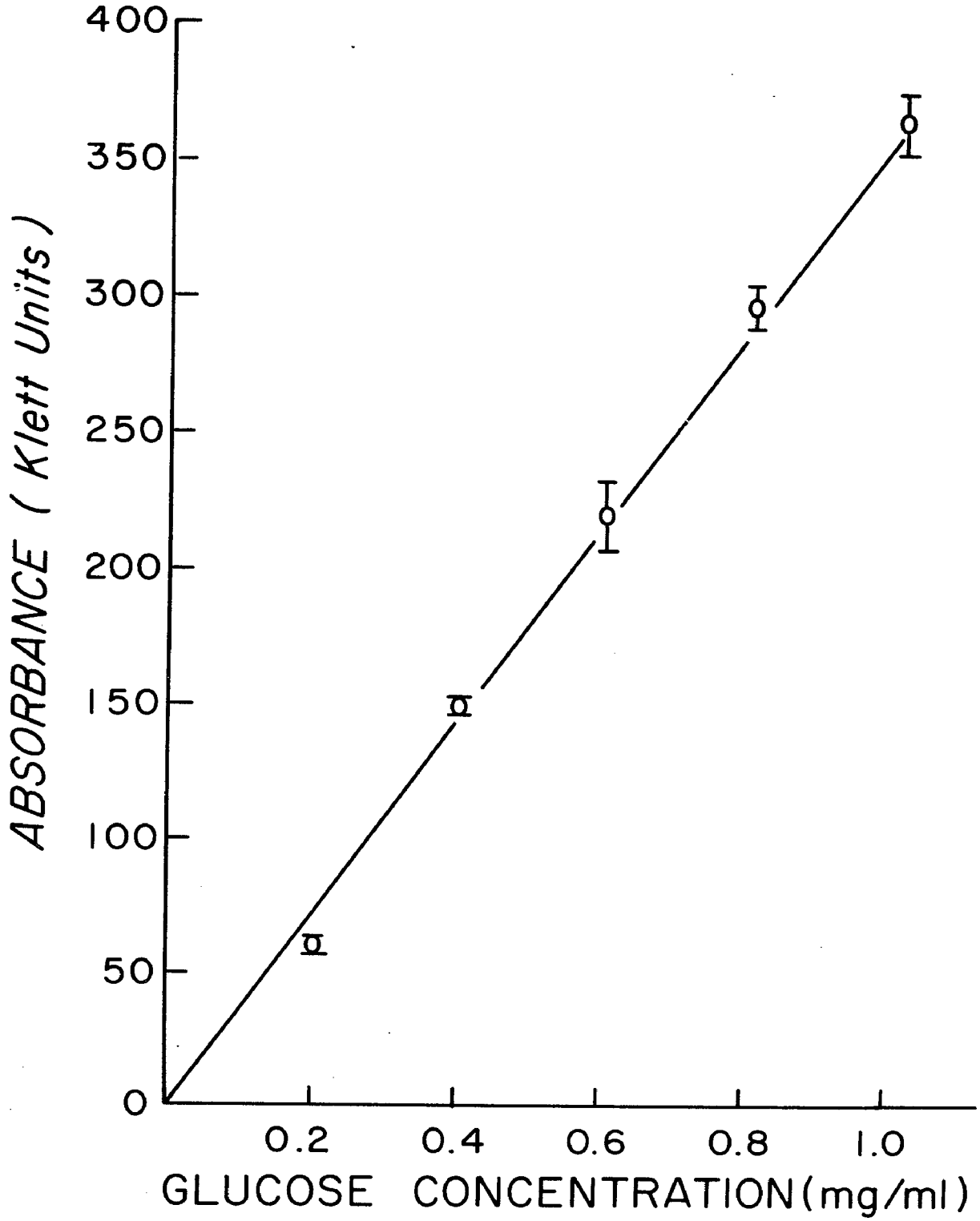
glucose, formed in a 15 minute digestion period at 37°C per milligram of saliva. (Saliva volume is expressed in milligrams so that the same units are used for saliva volume and tissue mass. The specific gravity of saliva is 1.0.) The values were computed from a standard curve, which related Klett units to glucose concentration, as shown in Figure 2. The slope shows an equivalence of 0.002822 milligram of glucose per Klett unit, with a standard deviation of ± 0.0002 mg/Klett unit. The value of 0.0028 milligram of glucose per Klett unit was used in calculation of all amylase activities.

The amylase activity of parotid glands was determined from the frozen tissue. The gland was ground in a glass mortar to which a small amount of sand was added. The ground gland was diluted to 10 ml with saline phosphate buffer and then centrifuged at 5°C for 20 minutes to separate the insoluble fraction. Fifty microliters of supernatant fluid were diluted to 5 ml with saline phosphate buffer. The saliva and gland samples were diluted with saline phosphate buffer to an extent sufficient to provide Klett readings that fell between 100 to 300 units, since the relationship to amylase activity is linear within this range. The amylase activity in parotid glands was expressed as milligram of reducing substance per milligram of gland wet weight.

Determination of secretory rates

In order to obtain salivary flow rates, saliva volumes were collected during timed periods, and glands were removed and

Figure 2. Standard curve for determination of glucose concentration. Each point represents mean ± standard deviation of 8 values. The average value of one Klett unit was 0.002822 mg of glucose, with a standard deviation of 0.0002 mg.


400
350
300
250
200
150
100
50
0
ABSORBANCE ( Klett Units )
0.2
0.4
0.6
0.8
1.0
GLUCOSE CONCENTRATION(mg/ml)

weighed after all volumes had been obtained. Flow rates could then be expressed in units of microliters per minute per gram of fresh tissue. From the salivary flow rates, and values for concentration of calcium, or activity of amylase, in the saliva, the secretory rate for calcium, or amylase, could be determined.

Treatment of data

Data from each rat were plotted as a time course of change in calcium concentration or amylase activity. The mid-point of the time interval of collection was used to determine the abscissa for each point. Successive points were connected by straight lines. For analysis of the data, values for calcium concentration or amylase activity were taken for the same time points from each curve, even if these did not coincide with the actual times samples were obtained. These interpolated values were used to calculate means, and standard errors of the means, for the selected time intervals. The difference between means was compared by standard Student's t-test. The difference was considered significant if P values were less than 0.05 (19).

# RESULTS

Submaxillary gland of rat was selected for initial investigation of the effects of diverse kinds of autonomic stimulation on calcium secretion for several reasons. First, it had been reported that calcium levels of the unstimulated submaxillary gland are unusually high, greatly exceeding that of other tissues (7, 8, 11). In addition, the course of calcium secretion in the rat submaxillary saliva evoked by some selected autonomic agents had also been reported (7, 11). Accordingly, experiments were initiated, first, to confirm the reported findings and, second, to examine the kinetics of calcium secretion in response to stimulation of the innervation to the gland. It was a particular objective of this work to determine effects on salivary secretion of calcium of stimulating the gland by way of its innervation, and not only by administration of autonomic drugs. Data on the role of the autonomic innervation in regulation of calcium secretion from submaxillary or parotid have not heretofore been available.

## Submaxillary gland and saliva

The data in Table 1 show that calcium level of submaxillary gland, as well as that of parotid gland, pancreas, and kidney of

TABLE 1. Calcium concentrations in various organs

| Organ | [Ca]* (mEq/kg wet weight) |
|---|---|
| Parotid gland | 12.97 ± 0.34 (20) |
| Submaxillary gland | 9.75 ± 0.52 (6) |
| Pancreas | 2.97 ± 0.09 (3) |
| Kidney | 3.03 ± 0.07 (3) |

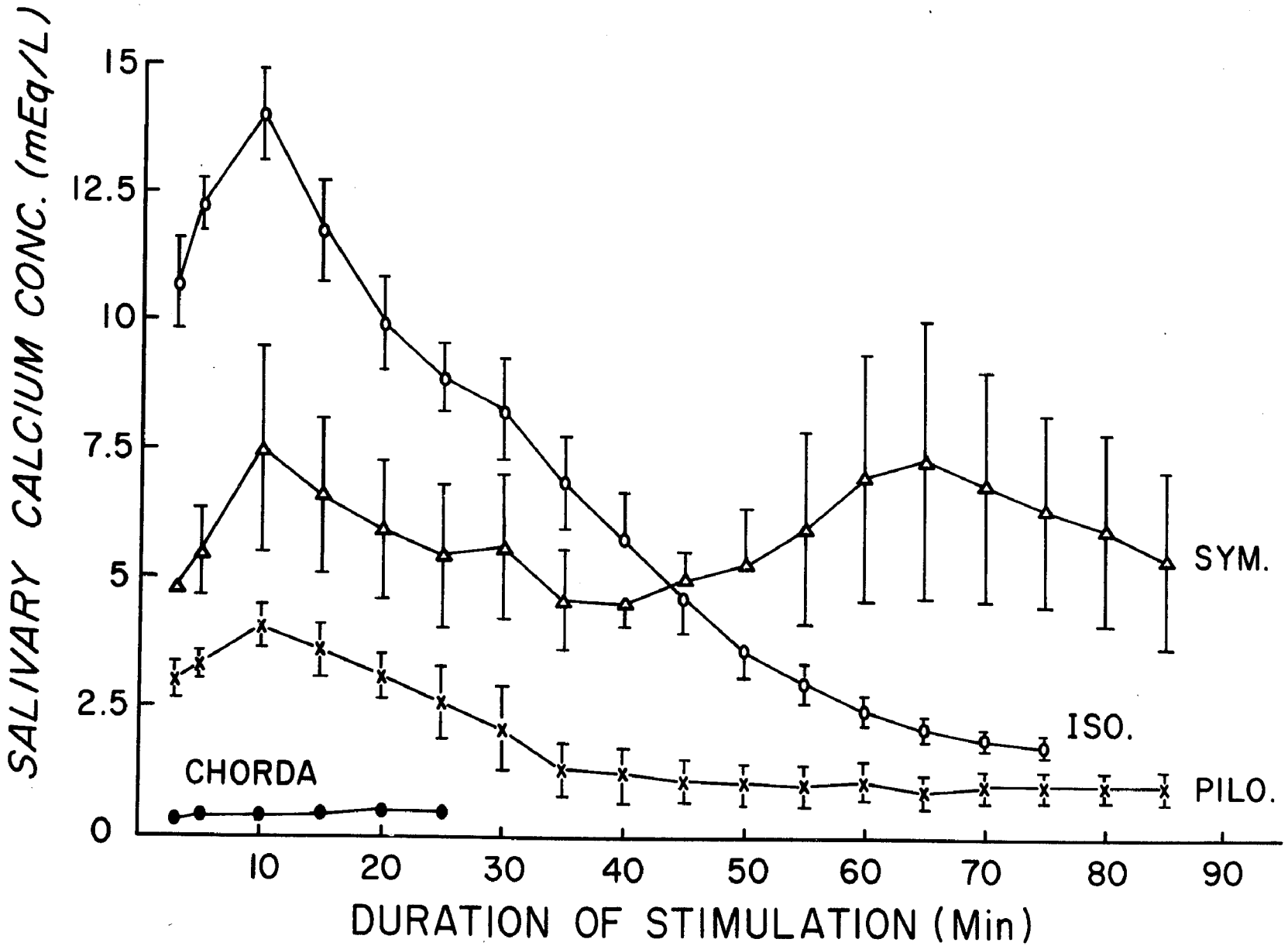
*The values are means ± standard error. The numbers in parentheses refer to the number of rats.

rats fasted 18 hours prior to organ removal. Calcium concentration of submaxillary gland at approximately 10 mEq/kg was 3 times as high as that of pancreas or kidney, but not as high as that of parotid. The difference between levels of parotid (13.0 ± 0.3 mEq/kg) and submaxillary (9.8 ± 0.5 mEq/kg) was statistically significant.

These findings were of interest for several reasons. First, they generally confirmed the earlier findings of Dreisbach (8) that, in rat, salivary gland shows a higher concentration of calcium than most other soft tissues, and, second, that parotid gland has an even greater concentration of calcium than submaxillary gland. Salivary calcium levels were then also examined to establish the validity of the reported values, obtained by stimulation with pilocarpine or isoproterenol, as well as to provide a point of reference for subsequent analysis of effects of nerve stimulation on the course of calcium secretion.

The data in Fig. 3 show the course of change in calcium concentration of submaxillary saliva evoked in response to various modes of stimulation. With pilocarpine, the calcium concentration of the saliva secreted first (at 3 min after pilocarpine injection) was 3.02 ± 0.33 mEq/l. A slight increase was observed at 10 minutes; this was followed by a statistically significant decrease ($P < .025$). A plateau was attained at 35 minutes, with concentrations of approximately 1.0 mEq/l. This was maintained for the additional 50 minutes of collection. The general course of these changes was consistent with findings previously reported by Dreisbach (7).

Figure 3. Calcium concentration in submaxillary saliva secreted in response to various modes of stimulation (SYM. = sympathetic nerve, ISO. = isoproterenol, PILO. = pilocarpine, CHORDA = chorda tympani nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


SALIVARY CALCIUM CONC. (mEq/L)
15
12.5
10
7.5
5
2.5
0
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
SYM.
ISO.
PILO.
CHORDA

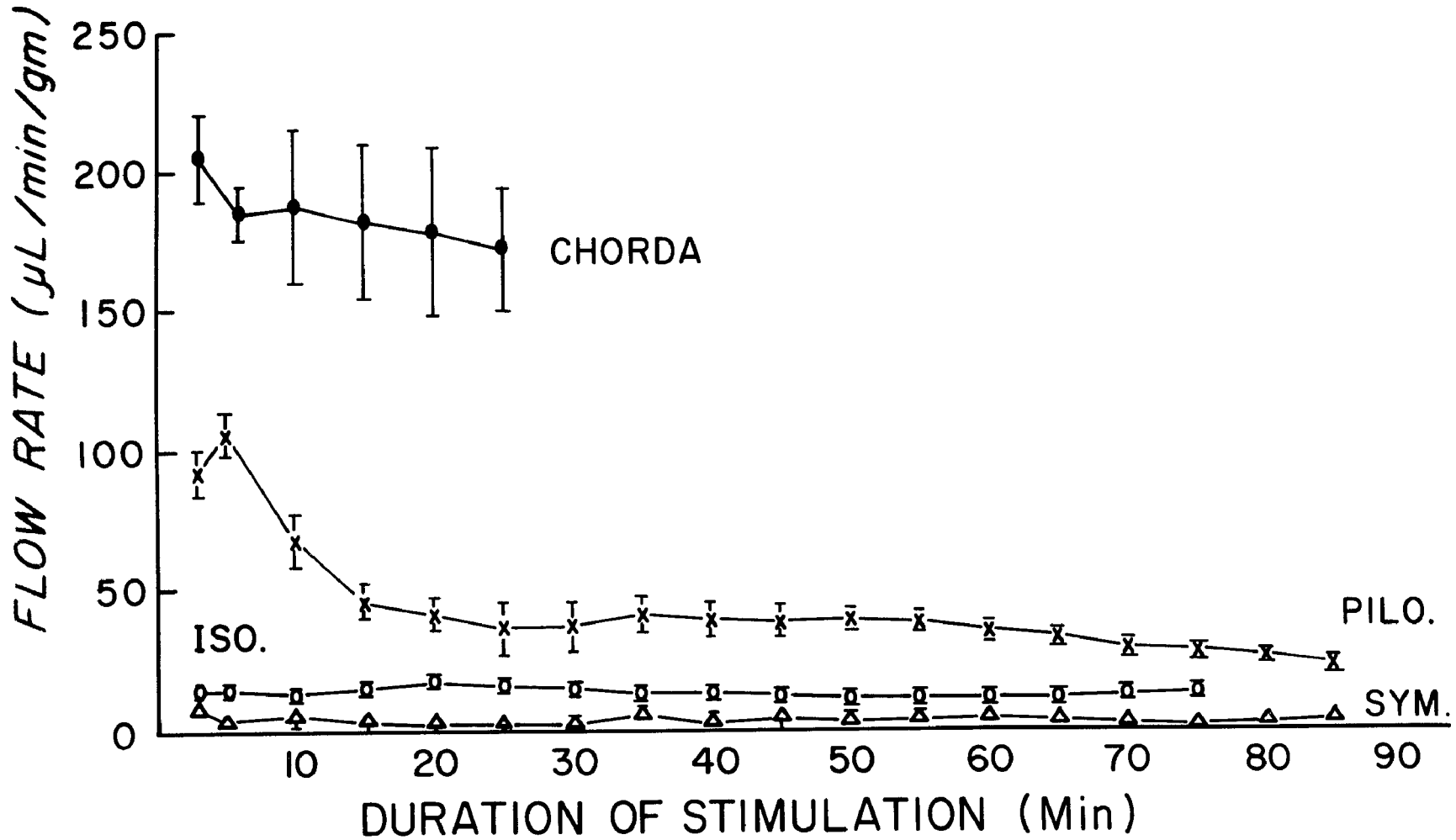
Calcium concentration of saliva evoked by isoproterenol was significantly higher than in that evoked by pilocarpine at all times during the 75 minutes of collection. However, by 75 minutes, the concentration of calcium in isoproterenol-evoked saliva was not much greater than in pilocarpine-evoked saliva. Thus, initially (after 3 minutes) levels were 10.7 ± 0.9 mEq/l in isoproterenol-evoked saliva and continued to increase until 10 minutes, when the high level of 14.0 ± 0.8 mEq/l was attained. Calcium concentration decreased continuously thereafter. At 20 minutes a statistically significant ($P < .005$) decrease of 29% from the maximal level was evident; between 30-35 minutes the magnitude of the decrease was nearly 45% from the maximal level; by 50 minutes, a 74% decrease from the maximal value was observed. These changes were statistically significant ($P < .05$). A plateau was not attained and levels recorded at 75 minutes were less than 2 mEq/l. Thus, the initial levels and course of change were considerably different when isoproterenol and pilocarpine stimulating effects were compared.

Direct stimulation of the autonomic innervation showed strikingly different results from those obtained with the autonomic drugs. Stimulation of the chorda tympani produced saliva containing the lowest levels of calcium recorded during these experiments (0.3 mEq/l; Fig. 3). Furthermore, there was no change in calcium level over the 25 minute period of collection (Fig. 3). Longer periods of stimulation were not attempted.

Stimulation of the postganglionic sympathetic fibers also presented some difficulty but collections for 85 minutes were made on at least two animals. The initial levels under these conditions of stimulation were higher (at about 5 mEq/1) than those observed with either pilocarpine or chorda stimulation but significantly less than those observed with isoproterenol stimulation. Mean values of 4 to 7.5 mEq/1 were recorded throughout. Statistically significant variations in concentration were not observed.

The differences in calcium concentration showed no consistent relationship to flow rate (Fig. 4). This was lowest with sympathetic nerve stimulation (between 2-6 µl/min/gm of gland for the entire 85 minute period of stimulation). Isoproterenol caused a slightly higher flow rate, 11-17 µl/min/gm, for this 75 minute period. Pilocarpine induced higher flow rates than sympathetic stimulation but rates were considerably less than those observed with stimulation of the cholinergic innervation. Thus, initial flow rates of pilocarpine-evoked saliva were 92.8 ± 9.2 µl/min/gm whereas those with chorda stimulation were 206 ± 15 µl/min/gm. Flow rate with chorda stimulation did not show any statistically significant changes with the 25 minute period of stimulation but with pilocarpine, flow rate after 15 minutes showed a significant ($P < .005$) decrease from initial levels of 50% (46.8 as compared with 92.8). This level was maintained for the next 50 minutes after which a further decrease occurred (to 23.0 ± 1.7 µl/min/gm). This level,

Figure 4. Changes in flow rate of submaxillary saliva with time, with four different modes of stimulation (CHORDA = chorda tympani nerve, PILO. = pilocarpine, ISO. = isoproterenol, SYM. = sympathetic nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


FLOW RATE (µL/min/gm)
250
200
150
100
50
0
CHORDA
PILO.
ISO.
SYM.
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)

maintained for the additional 40 minutes of stimulation, was higher than initial levels observed with either isoproterenol or sympathetic nerve stimulation.

Although flow rate with chorda nerve stimulation was at least 10 times greater than that observed with sympathetic nerve stimulation, the concentration of calcium secreted in each time period was always lower. However, the calcium output, in nEq/min/gm, during chorda stimulation was higher than that observed with sympathetic nerve stimulation (Fig. 5).

Parotid gland and saliva

The data in Fig. 6 show the concentrations of calcium in parotid saliva during periods of stimulation by various means. With supramaximal stimulation of the auriculotemporal nerve, calcium levels were initially high (11.8 ± 1.0 mEq/l) and remained at this level for the first 20 minutes of continuous stimulation. A decrease that was statistically significant ($P < .05$) was not evident until 40 minutes after initiation of stimulation, when calcium levels had dropped to 9.7 ± 0.7 mEq/l; between 60-90 minutes calcium concentration was close to 9 mEq/l; although this value was not significantly different ($P > .05$) from the 40 minute level, it represented an overall change of 29% from initial levels.

With pilocarpine stimulation, initial calcium levels were 9.4 ± 0.3 mEq/l and were significantly ($P < .025$) less than those observed with auriculotemporal stimulation. Within the first 15

Figure 5. Rate of calcium secretion by submaxillary gland (PILO. = pilocarpine, ISO. = isoproterenol, CHORDA = chorda tympani nerve, SYM. = sympathetic nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.

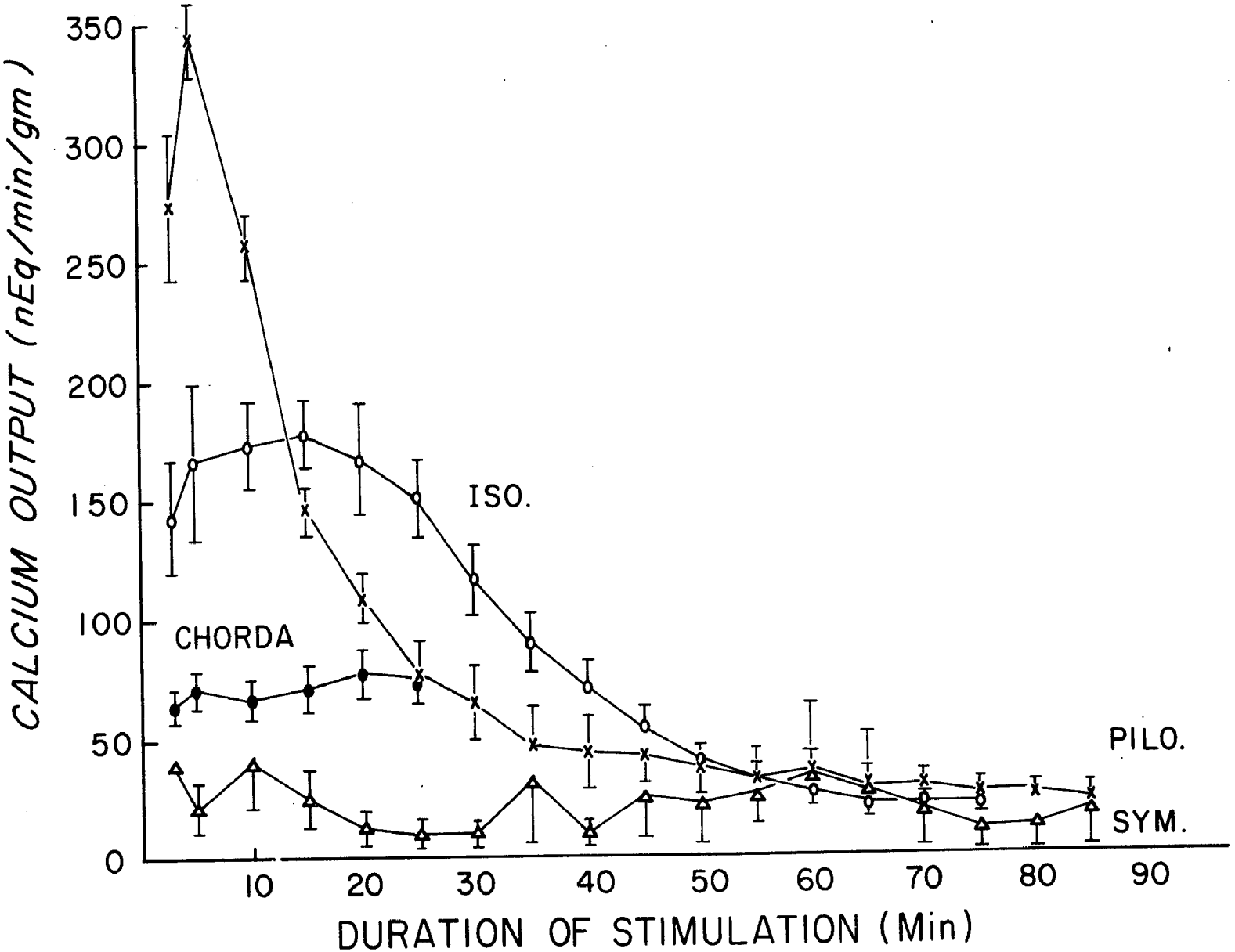
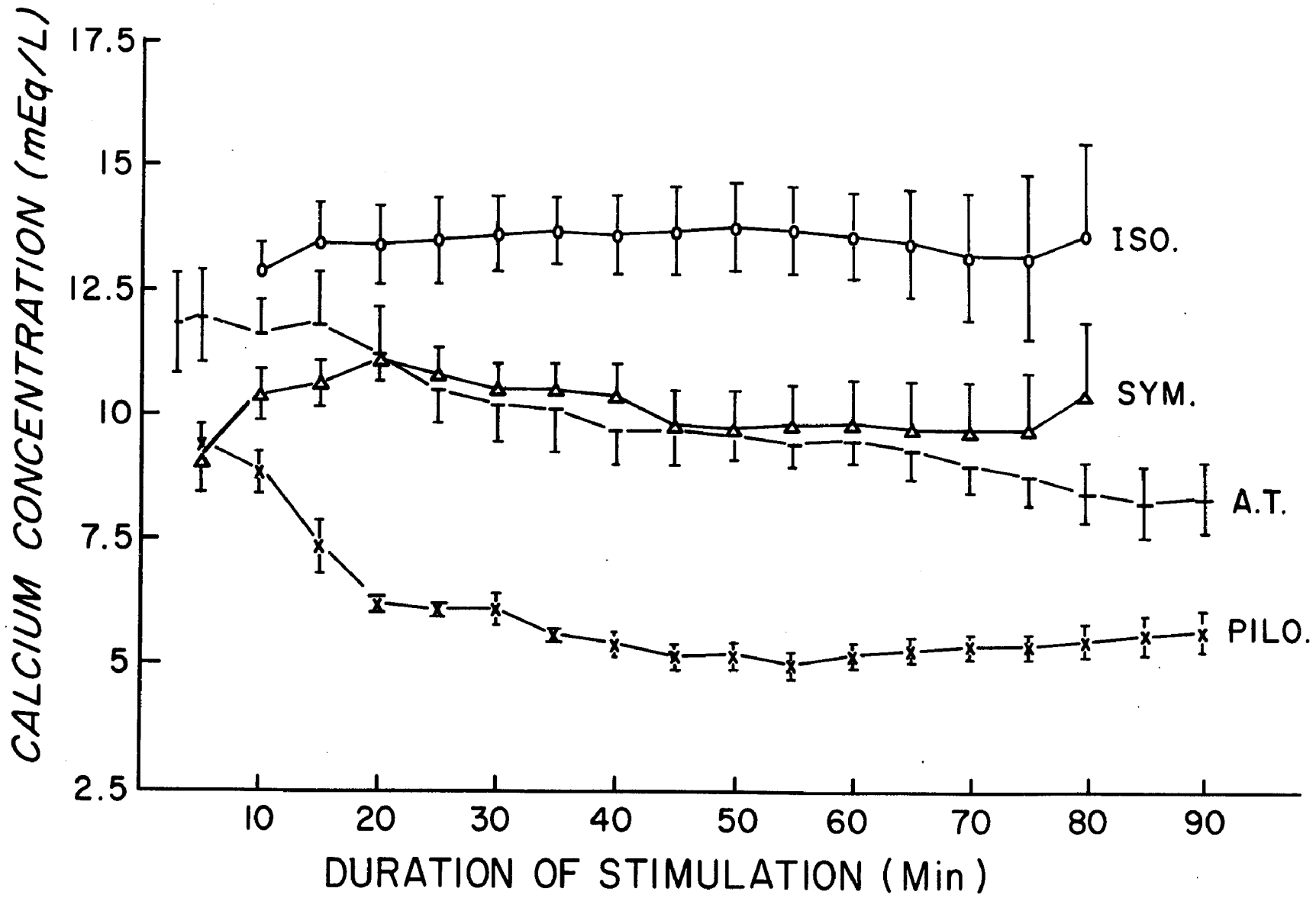

CALCIUM OUTPUT (nEq/min/gm)
350
300
250
200
150
100
50
0
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
ISO.
CHORDA
PILO.
SYM.

Figure 6. Changes in calcium concentration of parotid saliva during prolonged stimulation by various modes (ISO. = isoproterenol, SYM. = sympathetic nerve, A.T. = auriculotemporal nerve, PILO. = pilocarpine). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


CALCIUM CONCENTRATION (mEq/L)
17.5
15
12.5
10
7.5
5
2.5
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
ISO.
SYM.
A.T.
PILO.

minutes, a considerable drop occurred and 15 minutes after stimulation was initiated, calcium concentration, at 7.4 ± 0.5 mEq/1, was significantly different from initial levels ($P < .025$); by 20 minutes, levels dropped to 6.2 ± 0.07 mEq/1. A further decrease to 5 mEq/1 occurred between 35-55 minutes after initiation of stimulation; this plateau level was then maintained for the next 50 minutes.

With stimulation by isoproterenol a somewhat different course of change was observed. Saliva of sufficient amount for collection did not appear until 10 minutes after injection of the isoproterenol and the calcium level of this initially evoked saliva was approximately 13 mEq/1 (12.8 ± 0.6 mEq/1) and remained at this high level for the entire 80 minutes of stimulation (Fig. 6).

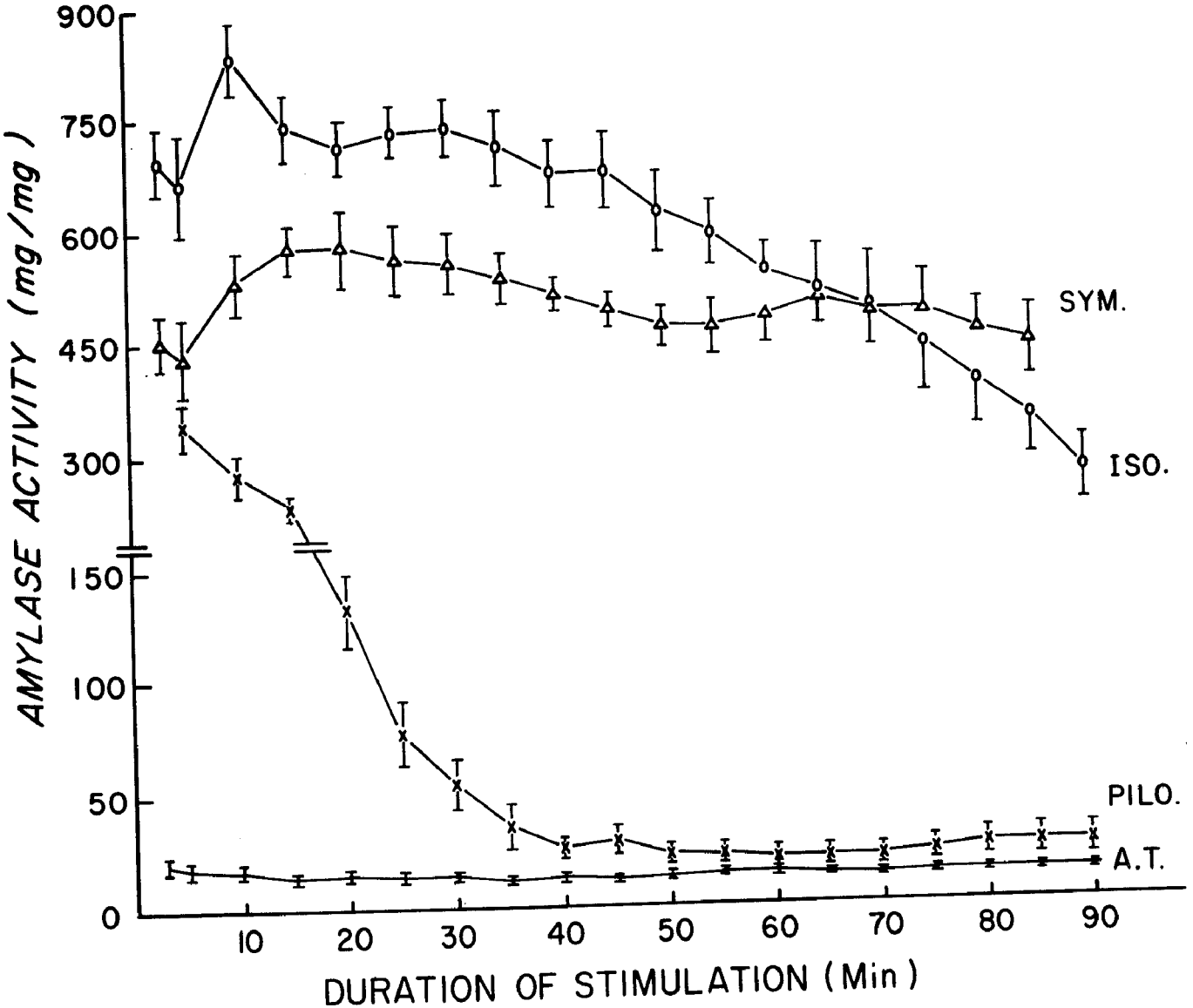
Concentration of calcium of saliva evoked by stimulation of the sympathetic innervation to the parotid was, in the first three minutes after initiation of stimulation, similar to that evoked with pilocarpine stimulation. Thus, in both instances, levels were approximately 9 mEq/1 (Fig. 6). A significant increase ($P < .05$) occurred within the next five minutes of sympathetic stimulation and reached a maximum level of 11.1 ± 0.4 mEq/1 at 20 minutes. For the duration of stimulation (the ensuing 60 minutes) there were no statistically significant changes, and calcium concentration at 20 and 80 minutes was approximately 10 mEq/1 (Fig. 6). Except for the initial phase of secretion

(3-20 minutes after initiation of nerve stimulation), the course of calcium secretion with sympathetic nerve stimulation was similar to that observed with stimulation of the auriculotemporal nerve. Furthermore, although the calcium levels with sympathetic nerve stimulation tended to be somewhat higher (10.5 ± 1.4 mEq/1) at the terminal point than those recorded with stimulation of the auriculotemporal nerve (8.5 ± 0.6 mEq/1), the difference between these two levels after 80 minutes of stimulation was not, however, statistically significant.

The course of amylase secretion in response to diverse autonomic stimuli is depicted by the data in Fig. 7. Supramaximal stimulation of the auriculotemporal nerve caused elaboration of saliva that was very low in amylase activity; in fact, initial amylase activity was at a level of only 18.5 ± 2.5 mg reducing substance per mg of saliva and there was virtually no change from this level for the entire 90 minutes of stimulation.

Pilocarpine stimulation, on the other hand, caused an initial secretion that had high levels of amylase (343 ± 26 mg/mg) but levels dropped markedly (to 232 ± 12 mg/mg) within the first 10-20 minutes. By 20 minutes, amylase levels had dropped further, to 131 ± 15 mg/mg. The sharp decrease continued so that by 25 minutes, levels were 77 ± 14 mg/mg and by 40 minutes, when a plateau occurred, amylase was at the same low level seen with auriculotemporal stimulation, i.e., 25 ± 3 mg/mg. The decrease for each interval between

Figure 7. Time course of change in amylase activity of parotid saliva in response to various modes of stimulation (ISO. = isoproterenol, SYM. = sympathetic nerve, PILO. = pilocarpine, A.T. = auriculotemporal nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


AMYLASE ACTIVITY (mg/mg)
900
750
600
450
300
150
100
50
0
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
SYM.
ISO.
PILO.
A.T.

15-25 minutes represented a statistically significant change from the preceding level ($P < .025$) and amounted in each case to about 40%.

Saliva evoked by isoproterenol had the highest level of amylase activity. Initially these were at least twice as high as levels observed with pilocarpine stimulation; a statistically significant increase ($P < .05$) from these levels of 662 ± 66 mg/mg to levels of 834 ± 46 mg/mg occurred within the first five minutes and thereafter concentration of amylase in the saliva remained at about 700 mg/mg for the next 35 minutes. There was not the sharp decrease with time which was observed with pilocarpine stimulation, but a reduction in amylase concentration occurred gradually over the next 50 minutes, with the level ultimately reaching a value of 269 ± 42 mg/mg. Thus, after 90 minutes of isoproterenol stimulation, amylase concentration of the saliva was only one-third of the initial levels; nonetheless, this final value was almost as high as that observed initially with pilocarpine stimulation.

Amylase activity in saliva with sympathetic nerve stimulation was initially very high (454 ± 33 mg/mg). Continuation of sympathetic stimulation led to a significant increase in amylase activity ($P < .05$) with a maximal activity of 575 mg/mg attained at about 15-20 minutes after initiation of stimulation. After that the level of amylase activity continued to go down for 30 minutes and remained at a plateau level between 450-515 mg/mg. The course of amylase activity from saliva evoked by sympathetic nerve and

isoproterenol showed significant differences from the beginning until 55 minutes after initiation of stimulation.

The course of change in amylase activity closely paralleled that of calcium concentration when sympathetic nerve stimulation, or pilocarpine, was employed. However, this was not the case with stimulation involving either the auriculotemporal nerve or administration of isoproterenol. While amylase and calcium concentration were both initially high with isoproterenol, increased over the first 10 minutes, and then tended to remain at a high plateau level for the next 30 minutes, there was a divergence thereafter. While calcium concentration of saliva remained at this plateau level, amylase dropped consistently for the next hour. With stimulation of the auriculotemporal nerve, there was no parallelism between amylase and calcium, either in the course of change or in the relative levels. Thus, while calcium concentration was initially relatively high (and not much less than levels observed with isoproterenol stimulation), amylase activity was very low, at an approximate level of only one-fortieth that observed with isoproterenol stimulation. Furthermore, amylase concentration did not change during the course of stimulation of the auriculotemporal nerve, and remained at this same low level throughout, while calcium concentration after the first 20 minutes decreased steadily from the initial high values.

Since there were these marked differences in absolute values as well as characteristics of the course of change, it was necessary

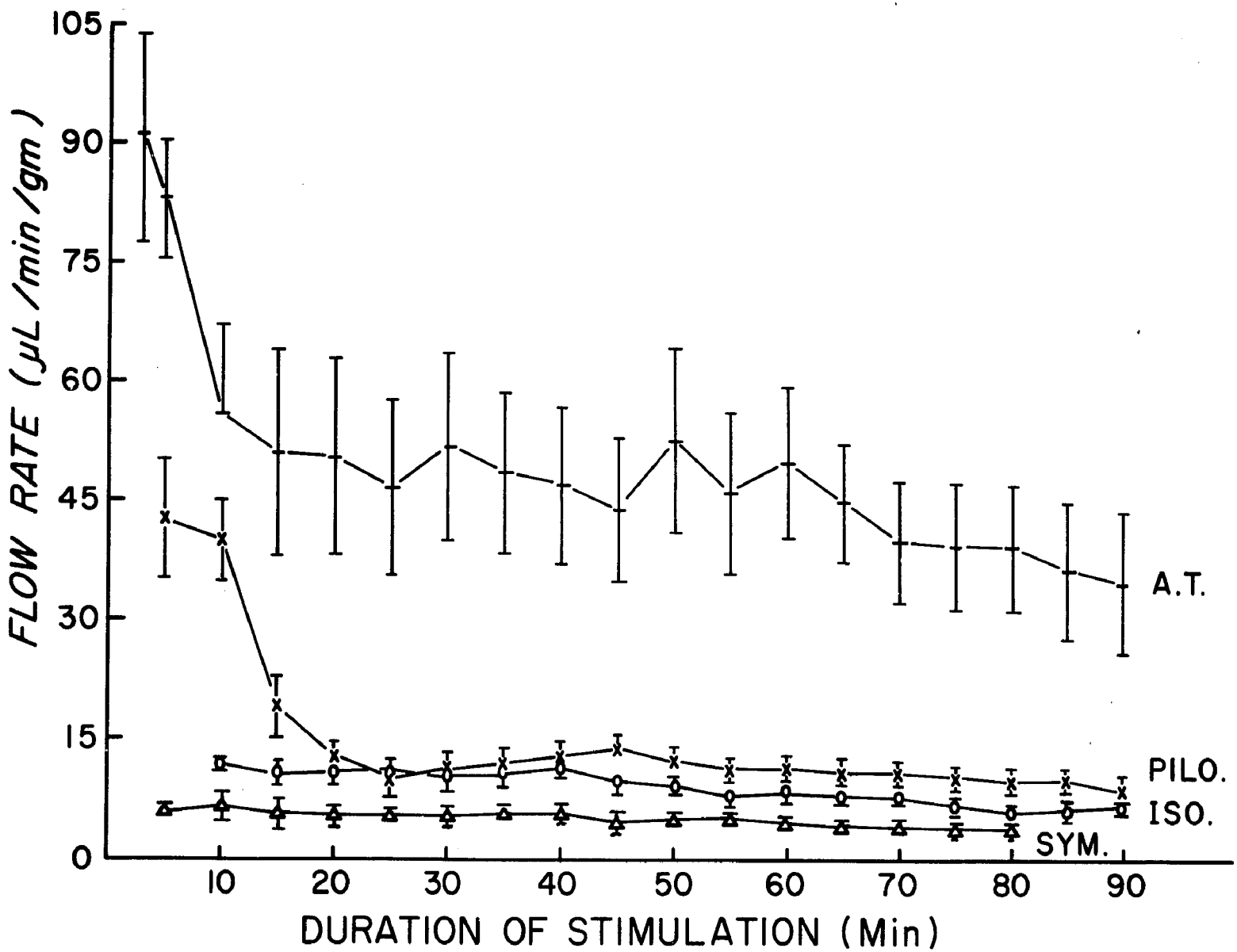
to examine other factors which could influence these parameters. Accordingly, flow rate characteristics with each kind of stimulation were also examined for the 90 minute interval of stimulation. Flow rate of saliva was highest when cholinergic stimulation was used to evoke the secretion. From the data in Fig. 8, it is clear that initial flow rates after stimulation of the auriculotemporal nerve was started were 91.3 ± 13.5 µl/min/gm of parotid gland but that a statistically significant ($P < .05$) decrease to 55.8 ± 11.4 µl/min/gm occurred within the first 10 minutes. Flow rate remained at about 50 µl/min/gm for the next 50 minutes, decreasing to less than 40 only within the last 20 minutes.

These flow rates were considerably higher than those of saliva evoked by pilocarpine, sympathetic nerve, or isoproterenol. The initially high values with pilocarpine were only one-half (42.7 ± 7.7 µl/min/gm) of the initial auriculotemporal values. There was a considerable (70%) decrease within the next 15 minutes, and a minimum level of 12.8 ± 1.8 µl/min/gm was recorded as early as 20 minutes after administration of pilocarpine. There was little change thereafter in flow rate. These changes with time were statistically highly significant ($P < .05$).

Flow rate with isoproterenol stimulation was initially low (11.7 ± 0.6 µl/min/gm) and remained at this low level throughout.

The lowest flow rate occurred with sympathetic nerve stimulation. Maximum stimulation of the sympathetic nerve produced saliva flow (6.5 ± 1.4 µl/min/gm) about half that of the flow evoked by

Figure 8. Changes in flow rate of parotid saliva with time, with four different modes of stimulation (A.T. = auriculotemporal nerve, PILO. = pilocarpine, ISO. = isoproterenol, SYM. = sympathetic nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


105
90
75
60
45
30
15
0
FLOW RATE (µL/min/gm)
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
A.T.
PILO.
ISO.
SYM.

isoproterenol administration. This flow rate remained almost constant until 80 minutes after stimulation.

In view of these marked differences in flow rate, the possibility existed that the rate of secretion of amylase and of calcium, are actually independent of the mode of stimulation and that the concentrations of these constituents depend solely on the flow rate. When the total amylase activity of saliva collected for the entire period of stimulation (usually 90 minutes) was measured, it was found that this total was equivalent to an activity of only 51 ± 8 mg/mg of gland wet weight, when the auriculotemporal nerve was stimulated (Table 2; Fig. 9). This amount was appreciably less than that observed with either isoproterenol or pilocarpine. With pilocarpine, the total activity for a 90 minute period amounted to 176 ± 40 mg/mg of gland wet weight; with isoproterenol, to 516 ± 48 mg/mg of gland wet weight; and with sympathetic nerve, to 238 ± 25 mg/mg of gland wet weight. In none of these instances, therefore, did flow rate seem to be an important factor regulating the marked differences in amylase secretion.

The role of flow rate in determining calcium concentration was also examined. In parotid saliva, the rate of calcium secretion did depend on the rate of saliva secretion. Calcium secretion was highest with auriculotemporal stimulation, at 41.6 ± 7.4 mEq/ 90 min/kg of gland wet weight. The amount secreted with isopro-

TABLE 2. Total output of amylase activity and calcium in rat parotid saliva with diverse autonomic stimulation (90 minutes duration).

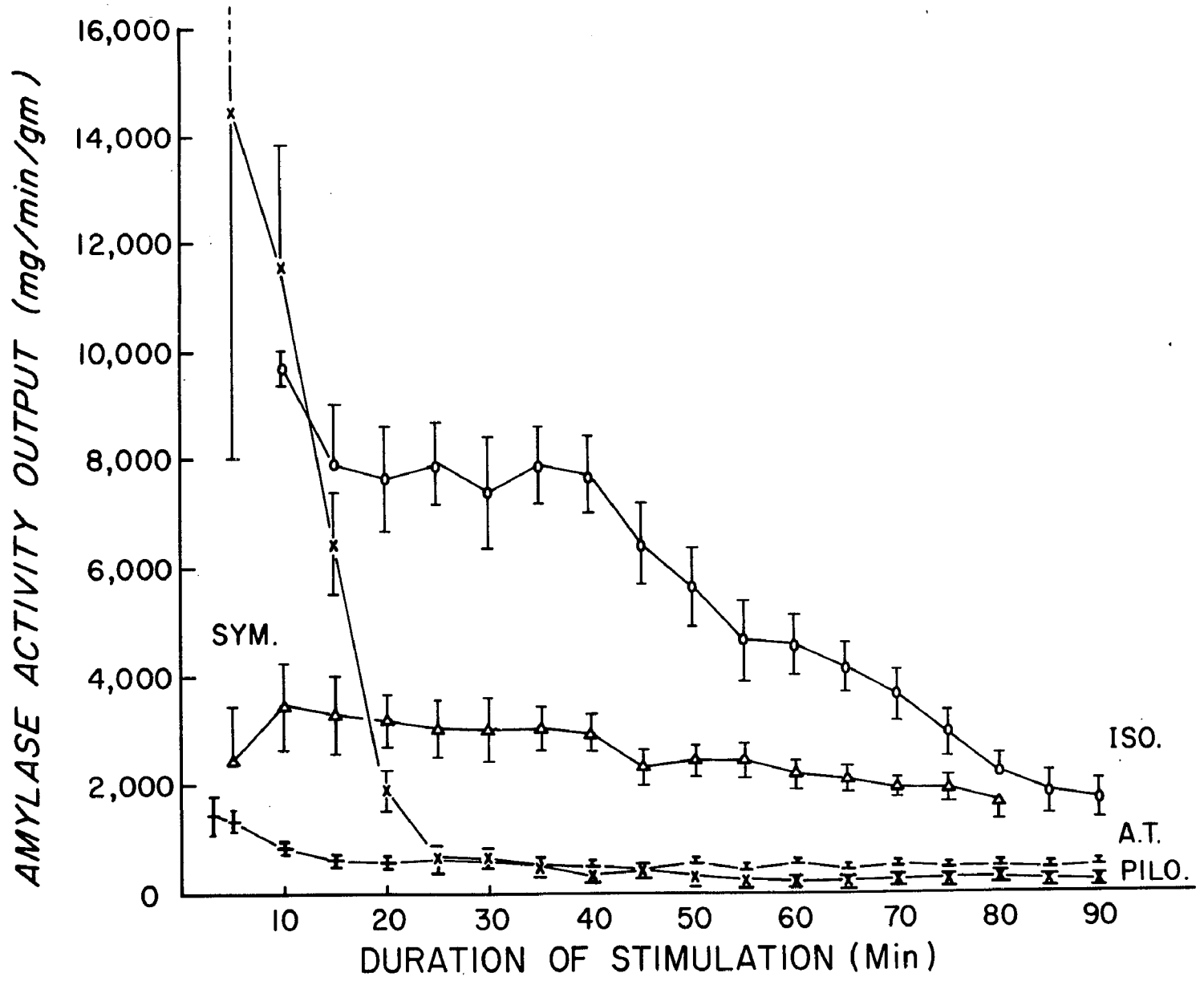
| Kind of Stimulation* | Total Amount in Parotid Saliva | |
|---|---|---|
| | Amylase Activity (mg/mg)† | Calcium (mEq/kg) |
| ISO. | 516 ± 48 (8) | 12.3 ± 0.1 (5) |
| PILO. | 176 ± 40 (4) | 7.9 ± 0.8 (3) |
| A.T. | 51 ± 8 (5) | 41.6 ± 7.4 (5) |
| SYM. | 238 ± 25 (5) | 4.7 ± 0.3 (5) |

Values are means ± standard error. Numbers in parentheses are number of rats in the experiment.

*ISO. = isoproterenol, PILO. = pilocarpine, A.T. = auriculo-temporal nerve, SYM. = sympathetic nerve.

†The amount of amylase activity or calcium output was determined on the basis of mg or kg wet weight of parotid gland.

Figure 9. Time course of amylase output into parotid saliva for four different modes of stimulation (PILO. = pilocarpine, ISO. = isoproterenol, SYM. = sympathetic nerve, A.T. = auriculotemporal nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.


AMYLASE ACTIVITY OUTPUT (mg/min/gm)
16,000
14,000
12,000
10,000
8,000
6,000
4,000
2,000
0
SYM.
ISO.
A.T.
PILO.
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)

terenol stimulation, at 12.3 ± 0.1 mEq/kg of gland wet weight, was nearly twice as great as that secreted in response to pilocarpine stimulation (7.9 ± 0.8 mEq/90 min/kg of gland wet weight), and almost three times greater than that secreted during sympathetic nerve stimulation (4.7 ± 0.3 mEq/90 min/kg of gland wet weight) (Table 2; Fig. 10).

Secretion of calcium or amylase can also be measured by determining levels of these moieties in the gland, following autonomic stimulation (Table 3). After 90 minutes of auriculo-temporal nerve stimulation, amylase levels of parotid were reduced from controls by about 29%; with the same duration of pilocarpine, sympathetic nerve stimulation, or isoproterenol stimulation, the reduction from levels of unstimulated gland was 46%, 63% and 89%, respectively. Thus, a very small reduction in gland amylase was induced with cholinergic nerve stimulation, and this agreed with the observed small output of amylase in saliva; a large reduction occurred with isoproterenol or sympathetic nerve stimulation and this also agreed with the observed high levels in the saliva. In case of pilocarpine, a possible discrepancy appeared to exist between the extent of glandular depletion of amylase and the amount which appeared in the saliva.

Attempts were made to account for the discrepancy observed with pilocarpine. Stimulation caused some change in water content of the gland and the percent of gland water following 90 minutes

Figure 10. Time course of calcium output into parotid saliva, evoked by different modes of stimulation (A.T. = auriculotemporal nerve, PILO. = pilocarpine, ISO. = isoproterenol, SYM. = sympathetic nerve). Duration of Stimulation, on the abscissa, denotes the duration of the period which followed after stimulation was started.

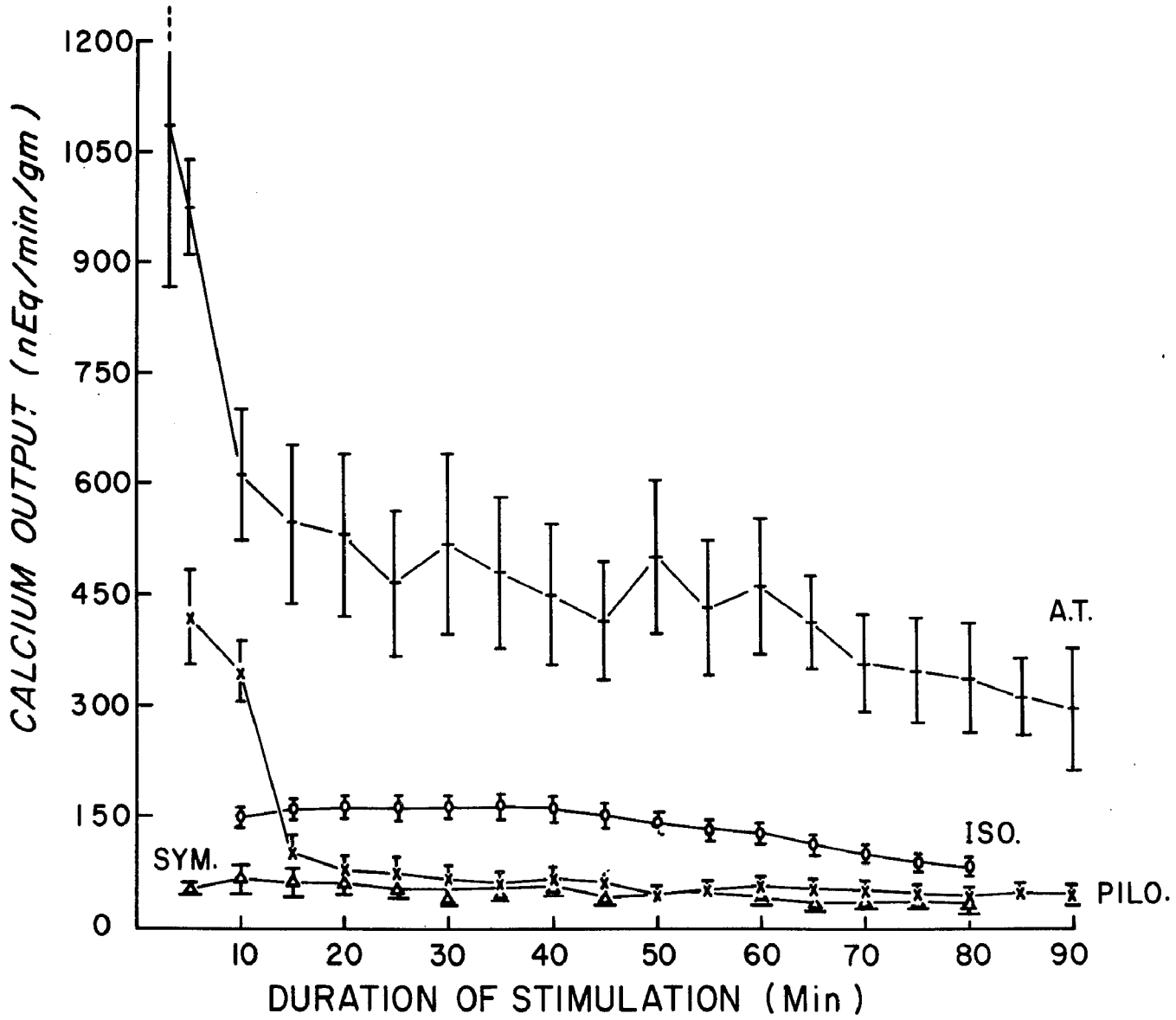

CALCIUM OUTPUT (nEq/min/gm)
1200
1050
900
750
600
450
300
150
0
10
20
30
40
50
60
70
80
90
DURATION OF STIMULATION (Min)
A.T.
ISO.
PILO.
SYM.

TABLE 3. Water content, amylase activity and calcium concentration of rat parotid gland with diverse autonomic stimulation.

| Kind of Stimulation$^{\Delta}$ | Rat Parotid Gland | | |
|---|---|---|---|
| | Water Content (percent) | Amylase Activity (mg/mg) | [Ca] (mEq/kg) |
| None | 70.6 ± 0.8 (20) | 537 ± 29 (11) | 13.0 ± 0.3 (20) |
| ISO. | 76.8 ± 1.5 (9) | 59 ± 12 (6) | 5.2 ± 0.4 (9) |
| PILO. | 73.3 ± 1.7 (5)* | 288 ± 23 (3) | 10.7 ± 0.7 (5) |
| A.T. | 74.3 ± 1.0 (8) | 380 ± 24 (4) | 11.3 ± 0.9 (8) |
| SYM. | 78.0 ± 2.0 (5) | 199 ± 16 (5) | 8.4 ± 0.2 (5) |

$^{\Delta}$In each case, duration of period after start of stimulation was 90 minutes (ISO. = isoproterenol, PILO. = pilocarpine, A.T. = auriculotemporal nerve, SYM. = sympathetic nerve).

Values are means ± standard error (per unit wet weight of gland); all values from experimental animals differ significantly from control ($P < .05$) except where indicated by *. The numbers in parentheses are number of rats.

of stimulation with pilocarpine or stimulation of the auriculotemporal nerve was 73.3 ± 1.7 and 74.3 ± 1.0, respectively. With isoproterenol, or stimulation of sympathetic nerve, percent water was, respectively, 76.8 ± 1.5 and 78.0 ± 2.0. Thus, while water content of glands following sympathetic, parasympathetic and isoproterenol was increased ($P < .005$) from control levels (70.6 ± 0.8), with pilocarpine stimulation, water content was not different from that of control ($P > .05$). Thus, the discrepancy in amylase levels with pilocarpine stimulation is not related to change in water content of the gland.

It is possible that the marked drop in amylase of glands stimulated by pilocarpine for 90 minutes is largely due to the unusually high amylase activity of the saliva secreted during the first 20 minutes of stimulation. This unusually high amylase is presumed to be due to a sympathetically-mediated effect which occurs when blood levels of pilocarpine are unusually high (35, 36). This would be the case soon after injection of pilocarpine in a single dose. Therefore, gland levels of amylase were measured after 20 minutes of pilocarpine stimulation. As shown in Table 4, the major drop in gland amylase did occur within the first 20 minutes. Therefore, the apparent discrepancy between the stimulatory effects of pilocarpine and auriculotemporal nerve on amylase depletion in the gland can be accounted for.

Calcium levels of parotid gland after 90 minutes of stimulation (Table 3) were also reduced. The biggest reduction occurred

TABLE 4. Water content, amylase activity and calcium concentration of rat parotid gland with diverse autonomic stimulation (time indicated).

| Kind of Stimulation | Rat Parotid Gland | | |
|---|---|---|---|
| | Water Content (percent) | Amylase Activity (mg/mg) | [Ca] (mEq/kg) |
| None | 70.6 ± 0.8 (20) | 537 ± 29 (11) | 13.0 ± 0.3 (20) |
| A.T. (20 min) | 72.2 ± 0.7 (6)* | 434 ± 21 (6) | 13.0 ± 1.0 (6)* |
| PILO. (20 min) | 72.4 ± 0.8 (6)* | 298 ± 21 (6) | 11.4 ± 0.3 (6) |
| PILO. (35 min) | 69.5 ± 0.8 (6)* | 360 ± 21 (5) | 11.5 ± 0.4 (6) |

Values are means ± standard error; all values from experimental animals differ significantly from control ($P < .05$) except where indicated by * (A.T. = auriculotemporal nerve, PILO. = pilocarpine). The numbers in parentheses are number of rats.

with isoproterenol and the decrease from 13.0 ± 0.3 mEq/kg of gland wet weight in unstimulated control glands to 5.2 ± 0.4 mEq/kg of gland wet weight in isoproterenol constitutes a 60% reduction in gland calcium concentration. Sympathetic stimulation produced a reduction of calcium of about 35%. With pilocarpine, only an 18% reduction was effected, and auriculotemporal stimulation caused only a 13% decrease; all of these changes, though small, were statistically significant. The output of calcium in the saliva during this 90 minute interval (Table 2) reflects this pattern of change with isoproterenol, sympathetic and pilocarpine stimulation but with auriculotemporal stimulation the changes are quite unexpected. The calcium output in saliva is three times greater than that found in isoproterenol-evoked secretion (41.6 ± 7.4 mEq/kg of gland wet weight as compared with 12.3 ± 0.1 mEq/kg of gland wet weight), yet there was little change in gland levels of calcium following 90 minutes of cholinergic nerve stimulation. The possibility that water changes could account for this were ruled out when comparisons of calcium were made on the basis of water content of the gland. These percent changes were not very different from those based on wet weight.

The amount of amylase secreted per minute in parotid saliva is shown by data in Fig. 9. During the initial period of stimulation, pilocarpine caused the highest output of amylase. Within 25 minutes after initiation of stimulation, the output decreased markedly, reaching levels of 671 ± 181 mg/min/gm of gland wet weight.

Amylase output at this point was at the same low levels observed with stimulation of the auriculotemporal nerve (535 ± 120 mg/min/gm of gland wet weight). In fact, the output with parasympathetic nerve stimulation was low (500-1500 mg/min/gm of gland wet weight) for the entire 90 minute period of stimulation. Thus, for the period 25-90 minutes, the amylase output was similar for the two kinds of parasympathetic stimulation. With the two kinds of sympathetic stimulation, however, marked differences existed. Isoproterenol caused an initially high output of amylase that was only slightly less than the initial maximal found with pilocarpine. Amylase output was high for at least 40 minutes following administration of isoproterenol, and therefore, total amylase output for the 90 minute period was, of the four kinds of stimulation employed, highest with isoproterenol. The amylase output with sympathetic nerve stimulation was, except for the last 20 minutes of the 90 minute period of stimulation, very much less than that evoked with isoproterenol. Initially, the output was only one-fifth that of isoproterenol levels; initial output was 2447 ± 1047 mg/min/gm of gland wet weight, nonetheless twice as high as that found with stimulation of the auriculotemporal nerve.

The amount of calcium secreted per minute in parotid saliva is shown by data in Fig. 10. Of the four modes of autonomic stimulation employed, the highest calcium output was produced by stimulation of the parasympathetic nerve. The maximum output occurred 3 minutes

after initiation of stimulation. There was a sharp decrease in output in the next 2 minutes and within the next 10 minutes following stimulation, a further drop occurred. No further change occurred for the next 45 minutes, but thereafter a progressive decrease was observed.

The initial calcium output in saliva evoked by pilocarpine was very high (421 ± 65 nEq/min/gm of gland wet weight), but within 15 minutes, the amount of calcium secreted per minute decreased to a very low level (112 ± 25 nEq/min/gm of gland wet weight); with sympathetic nerve stimulation, calcium output was initially low (55 ± 7 nEq/min/gm of gland wet weight), and remained at this low level throughout the 80 minute period of stimulation. The calcium output with isoproterenol stimulation was initially twice as great as that observed with sympathetic nerve stimulation; this difference persisted for at least 60 to 70 minutes.

The calcium output following the 90 minute period of parasympathetic stimulation was about five times greater than the output evoked with stimulation of sympathetic nerve or pilocarpine, and about three times greater than that produced in response to isoproterenol stimulation.

# DISCUSSION

This work provides additional evidence to support the view that the composition of saliva is related to the kind of autonomic stimulation used to evoke the secretion. Additionally, evidence also is provided showing that gland levels of calcium and secretory protein are altered as a consequence of autonomic stimulation and that the nature and extent of the alteration is related specifically to the kind of autonomic stimulation. Secretory characteristics can therefore be determined in two ways: in terms of the composition of the secretion, and also in terms of the changes that occur in the composition of the gland. This, for example, is the case with rat parotid amylase; the level of activity of this digestive enzyme in the saliva and the degree of its depletion in the gland both reflect the kind of autonomic stimulation used to evoke secretion. Characteristically, stimulation with isoproterenol, or by way of the sympathetic innervation, results in the highest levels of amylase in the saliva, in the greatest amount of amylase secreted per unit time, and in the greatest depletion of amylase from the gland cells. This is true even for extended periods of stimulation, of up to 90 minutes.

Cholinergic stimulation, either through the parasympathetic innervation or by low doses of pilocarpine, characteristically causes only a very modest reduction in gland amylase, even when the stimulation is prolonged, and the evoked saliva has very low levels of amylase. The difference between these levels and those induced with isoproterenol stimulation are 30-40 fold. Even with the large volumes of fluid secreted with cholinergic stimulation, the total amount of amylase secreted is only one-tenth of that elaborated with isoproterenol stimulation. Furthermore, even during the initial period of stimulation, when the maximal differences in all parameters are evident, while auriculotemporal-stimulated flow is five times as great as isoproterenol stimulated flow, amylase levels in the isoproterenol-evoked saliva are 30-40 times greater. At least this 5-fold difference persists throughout the period of stimulation, up to the 90-minute limit tried. Consistent with this is the very small degree of depletion of gland amylase during cholinergic stimulation, as compared with depletion in response to isoproterenol.

With high doses of pilocarpine several apparent discrepancies become evident. Thus, amylase activity in saliva evoked by large doses of pilocarpine is higher than in saliva evoked by stimulation of the parasympathetic innervation, and the course of change, with time, is different. With pilocarpine, amylase activity in parotid saliva is particularly high only during the first 20-30 minutes of secretion, and then falls over the next 60 minutes, to approach parasympathetically-evoked levels.

The initial high level of amylase activity in pilocarpine-evoked parotid saliva has been shown to be due to a sympathetic action of pilocarpine, resulting from stimulation of muscarinic receptors in cells of the superior cervical ganglion (20, 36). It is also this sympathetic action which undoubtedly accounts for the marked emptying of gland amylase seen during the first 20 minutes of stimulation. As previous work showed (36), when the superior cervical ganglion is surgically removed just prior to pilocarpine, amylase levels in the pilocarpine-evoked saliva are very low, and are then indistinguishable from the very low levels found in saliva evoked by electrical stimulation of the cholinergic innervation to the parotid. The gland emptying, under these conditions, is also small and resembles that caused by stimulation of cholinergic innervation. These findings are important in reconciling the differences in stimulatory effects induced by the parasympathomimetic drug, pilocarpine, and those induced by stimulation of the parasympathetic innervation. The pronounced effect on amylase secretion apparently can be attributed to a β-adrenergic action of pilocarpine (20, 36).

The decrease in amylase level in the saliva, seen after the first 20 minutes of stimulation, following the injection of a large dose of pilocarpine, probably is attributable to at least two factors: the gradual reduction of blood levels of pilocarpine to a range where the sympathomimetic effect becomes less prominent, and the decrease in gland levels from which the salivary activity is derived.

The data all support the view that while stimulation of either cholinergic or adrenergic receptors causes secretion of amylase into the saliva and reduction of gland stores of amylase, adrenergic stimulation is far more effective than cholinergic in this capability. Indeed, even when a parasympathomimetic agent appears to have a greater capacity for inducing amylase secretion than is normally observed with stimulation of the parasympathetic innervation, the enhanced ability stems from a sympathetic source. The data also show that cholinergic stimulation results in far greater volumes of secretion but that regardless of this, sympathetic stimulation causes a greater depletion of amylase from the gland and a greater total output of amylase into the saliva than does parasympathetic. With auriculotemporal nerve stimulation and isoproterenol stimulation, which are assumed to be characteristic of each kind (parasympathetic and sympathetic) of autonomic stimulation, there exists a parallelism between depletion of gland amylase and secretion into the saliva. Furthermore, in each case, the amount secreted per unit of time remains constant with auriculotemporal stimulation and fairly constant (at least for 50 minutes) with isoproterenol stimulation. The difference between the two is 30- to 40-fold, at least for the first hour, and at least 10-fold thereafter. This greater effect of the sympathetic agent can only be attributed to a difference in receptor efficacy since flow rate differences, which are in the opposite direction, would only add

further evidence to the view that adrenergic receptors are far more potent than cholinergic in inducing amylase secretion.

While previous work has shown that cholinergic as well as adrenergic stimulation can cause amylase secretion but that adrenergic stimulation is the more effective in this regard, the course of the secretion has not been shown, the total output has not been determined, and a precise determination of the parallelism between gland emptying and secretion into saliva has not been made. Furthermore, while amylase secretion has been shown to be independent of flow rate (35), and that differences between various kinds of autonomic stimulation thus do not depend on flow rate differences, the present data established this point with direct evidence. Critical evidence of these relationships was obtained by examining the changes with time in secretion of amylase and flow rate and levels of gland amylase. Such data are especially important to establishment of a cholinergic role in regulation of amylase secretion, and that this role is a separate one from that of adrenergic receptors. Some investigators have suggested that β adrenergic receptors not only have the more important role in regulation of amylase secretion but that these receptors have virtually the exclusive role in mediating secretion of amylase (2, 44). Not only the present data, but earlier findings (36) to the effect that the sympathomimetic action of pilocarpine on amylase secretion can be blocked by removal of the superior cervical ganglion, or by administering adrenergic antagonist

drugs, leaving secretion which is typically cholinergic-like, demonstrate that a true cholinergic stimulation of amylase secretion can occur.

Electrolyte secretion has also been shown to be related to the kind of autonomic stimulation employed to evoke the saliva (36, 38, 53). $K^+$ for example is known to be high in saliva following sympathetic stimulation but considerably lower when parasympathetic stimulation is employed (36, 37, 53). $HCO_3^-$ also shows a similar pattern. $Na^+$ on the other hand is higher in some parasympathetically evoked salivas than in sympathetically evoked salivas (53). Calcium, like K and $HCO_3$, was expected to be high with sympathetic and low with parasympathetic stimulation. $Ca^{++}$ concentration of sympathetically evoked saliva is higher than that evoked with cholinergic stimulation, but the difference between the two is very small (less than 8%). Thus, with isoproterenol stimulation calcium was 13 mEq/l, but with sympathetic nerve stimulation it was 11 mEq/l, a value virtually identical to that of saliva obtained with parasympathetic nerve stimulation, at 12 mEq/l. The high level was however maintained with isoproterenol stimulation; in fact, a small increase occurred with time, and while high levels were maintained for at least the first hour, there was a decrease within 90 minutes with auriculotemporal or sympathetic stimulation. Pilocarpine stimulation showed much lower levels than either of these. Since both cholinergic and adrenergic stimulation result

in higher levels than these, it is difficult to account for the pilocarpine levels. Preliminary experiments show that calcium is directly dependent on flow rate, when cholinergic stimulation is used. Therefore, the lower values of salivary calcium recorded with pilocarpine can be due to flow rate differences. It should be noted, parenthetically, that this relationship does not seem to hold for sympathetic or sympathomimetic stimulation, where no change in salivary [Ca] has been seen in relation to rate of flow.

The similarity between calcium levels of isoproterenol- and auriculotemporal-stimulated salivas cannot however be attributed to flow rate since flow rate is low with isoproterenol and high with auriculotemporal nerve stimulation, but calcium is similarly high with both, and calcium concentration drops as flow rate is decreased during auriculotemporal stimulation. The similarity must be attributed to the separate effect of each kind of autonomic receptor.

Gland levels of calcium are also reduced following autonomic stimulation. Stimulation with the sympathetic agent isoproterenol causes the most pronounced decrease (60%) and auriculotemporal caused only a slight decrease (13%). The decrease caused by pilocarpine is only somewhat greater than that caused by cholinergic nerve stimulation, but less than that caused by sympathetic nerve stimulation. The output of calcium in the saliva during this 90 minute period of stimulation, on the other hand, does not parallel

the changes in gland levels. As anticipated, on the basis of the high levels of calcium in the saliva and the high flow rate, calcium output in the saliva evoked by stimulation of the parasympathetic innervation is very high; the output with isoproterenol stimulation is only one-third as great as this, but again, since flow rate was for much of the 90 minutes about one-third that of auriculotemporally evoked saliva, even though the calcium levels were similar for a good part of this time, this finding was anticipated. There is not good agreement between the levels of calcium remaining in the gland following 90 minutes of stimulation and the observed output in the saliva. Thus, with auriculotemporal stimulation, although calcium concentration of saliva is very high initially (12 mEq/1), remains relatively high throughout, and calcium output is great, there is very little change in calcium levels of the gland.

Furthermore, there is a smaller decrease in gland levels following pilocarpine stimulation than would be anticipated from examination of salivary calcium levels and total output in the saliva. Thus, only an 18% decrease in gland levels was recorded, compared with a 60% decrease after stimulation by isoproterenol, although the output of calcium in pilocarpine-evoked saliva was one-half that found with isoproterenol stimulation. Gland levels of calcium were examined at 20 and 35 minutes in an attempt to account for the inconsistencies, especially with auriculotemporal stimulation. There was the possibility that depletion occurred within the first 20 minutes

or so, and that reaccumulation of calcium in the gland occurred rapidly, at least when continuous cholinergic stimulation was delivered. However, after 20 minutes, there is no measurable change in gland levels of calcium with auriculotemporal stimulation; with pilocarpine, the change is about the same as that observed at 90 minutes (about 12%). Apparently, then, the changes in calcium in the saliva are not reflected in changes in the gland. It still remains possible that cholinergic stimulation causes a rapid re-uptake of calcium as well as secretion of calcium. Radioactive tracer work must be done to clarify this point.

Thus, while the effectiveness of diverse autonomic stimuli in changing net calcium is the same as for gland amylase, this is not true regarding secretion of calcium in the saliva. There is no real distinction between effectiveness of sympathetic versus parasympathetic stimulation in causing secretion of calcium in saliva. As a matter of fact, not only are the initial levels very similar but the output with parasympathetic nerve stimulation is at least three times greater.

In addition to the role of autonomic nerve stimulation in regulation of secretion of amylase and of calcium, individually, the present work explored the relationship between secretion of the two. Wallach and Schramm have shown that the two moieties are secreted in parallel from rat parotid slices when epinephrine is used as the stimulating agent and they believe this parallelism

could result from binding between the enzyme and calcium and packaging of these two components in the same secretory vesicles (52). However, these data were obtained only under in vitro conditions and only when catecholamines were used as the stimulating agent. The present work provides data showing the relationship between secretion of calcium and amylase under in vivo conditions, when diverse autonomic stimuli are employed, including the more nearly physiological condition of nerve stimulation. My data do not support the thesis that amylase and calcium are secreted in parallel under all conditions of stimulation. Thus, when secretion is measured in terms of disappearance from the gland, there does exist a degree of parallelism between secretion of amylase and calcium, since the extent of the depletion of the two is generally similar for any one kind of stimulation.

Even with regard to glandular depletion, however, the results of balance studies indicate that the parallelism between depletion of calcium and of amylase may be fortuitous, at least when parasympathetic stimulation is used, and that uptake of calcium into the gland during stimulation accounts for the apparent low level of depletion.

When the secretion of these substances into the saliva is examined, a parallelism between secretion of calcium and amylase is not so apparent. While amylase activity and calcium in saliva evoked by isoproterenol are both initially high, amylase levels

in the saliva decrease with time whereas calcium levels do not. This suggests that in vivo the mechanism for secretion of calcium may differ from that for amylase, even with β-adrenergic stimulation. The effect may also be interpreted in another way: that calcium and amylase are secreted in parallel for as long as levels of the two in the gland are adequately high; when a significant gland depletion occurs, the parallelism disappears. The disappearance of the parallelism and maintenance of continued high level of calcium in the saliva (possibly due to enhanced re-uptake) suggest that all preformed vesicles are depleted; secretion of the two (amylase and calcium) may then occur by another route (not packaged together).

The most conspicuous instance of a lack of parallelism between secretion of amylase and calcium is that involving stimulation of the parasympathetic nerve. Under these circumstances, very low levels of amylase are secreted into the saliva at a constant rate; however, the levels of calcium in the saliva are very high, and do not differ much in magnitude from the high calcium levels found in isoproterenol- and sympathetic-stimulated saliva. Furthermore, in this situation salivary calcium concentration ultimately is decreased, whereas salivary amylase concentration is not changed. The total amylase secreted is only a small fraction of the total gland pool available, about one-tenth that available, and this may account for the fact that

amylase concentration does not change throughout. However, calcium of saliva decreases with time whereas gland levels are hardly altered. A very active calcium re-uptake mechanism is thus suggested by these data also. It is possible, however, that calcium and amylase are each secreted by different processes when cholinergic instead of adrenergic stimulation is used. At any rate, the appearance of the two in the saliva does not follow a parallel course as far as magnitude of the concentrations or secretory rates are concerned.

The most convincing evidence of a parallelism between secretion of amylase and calcium is that obtained with pilocarpine and sympathetic nerve stimulation. Calcium and amylase are moderately high initially and both follow the same course of change with time, in pilocarpine evoked saliva, decreasing markedly within the next 20 minutes and reaching plateau levels at about 40 minutes.

All of the evidence thus points to the conclusion that, especially in parotid gland, calcium and protein (here, amylase) are not necessarily secreted in parallel. The kind of stimulation employed markedly affects the way in which the two are secreted. When cholinergic receptors are activated, only small amounts of enzyme are released; when β adrenergic receptors are activated, large amounts of enzyme are released. But large amounts of calcium are released with activation of either kind of receptor. Possibly, these differences are related to the particular nucleotide involved in mediating receptor activity. Cyclic AMP has been implicated as the second messenger in adrenergically induced

secretion of amylase and calcium; some evidence suggests that cyclic GMP may be involved in cholinergically-mediated secretion of calcium and amylase (46). Each of the nucleotides may exert the same effect in causing calcium release; however, the action in release of amylase may be significantly different. The present data certainly suggest some degree of separation in secretion of the two. The separation may be even more extensive than is presently apparent since re-uptake and ductal transfer may, during the period of stimulation, occur with calcium but not to significant extent with amylase (8, 18, 22).

Amylase is normally absent from submaxillary glands of rat, and levels determined on unstimulated glands simply reflect amylase content of the blood in the gland (41). With autonomic stimulation, there is an increase in these levels that cannot be attributed to blood levels but may reflect a concentration from the blood (41). Since amylase is, however, normally not present in submaxillary, only parotid could be used for analysis of the relationship between secretion of calcium and amylase. Nonetheless, since submaxillary gland has such a high level of calcium, its response to diverse autonomic stimulation was analyzed to see if autonomic stimuli produced identical effects, regardless of the kind of salivary gland. Results were surprising. There is a sharp contrast in effects obtained with stimulation of the parasympathetic innervation to the parotid and those obtained with

stimulation of the parasympathetic innervation to the submaxillary. Calcium concentration of cholinergically-evoked submaxillary saliva is extremely low, and values of less than 1 mEq/l were uniformly recorded, contrasting markedly with the high levels found in parotid saliva obtained under conditions of cholinergic stimulation. Furthermore, the levels obtained with isoproterenol stimulation were as high as those obtained with isoproterenol stimulation of parotid, and were 15-fold greater than the chorda values. With stimulation of the sympathetic innervation, levels were found to be only half those obtained with isoproterenol stimulation, unlike parotid where both types of adrenergic stimulation gave more comparable effects. Possibly, sympathetic nerve stimulation of submaxillary involves stimulation of $\alpha$ as well as $\beta$ receptors, since calcium levels are much less than those observed with the $\beta$ adrenergic agent, isoproterenol. On the other hand, with parotid, calcium levels with sympathetic nerve stimulation and isoproterenol stimulation are very similar, suggesting involvement of only $\beta$ adrenergic receptors in this case. The high calcium levels in parotid saliva evoked with stimulation of the parasympathetic nerve present an unusual condition, and one which deserves further investigation.

## SUMMARY

1) The course of amylase and calcium secretion into saliva and depletion of these two moieties from parotid glands is described by these experiments.
2) Initial levels and the course of change are found to depend on the kind of autonomic stimulation used to effect secretion.
3) Amylase and calcium levels following stimulation with adrenergic drugs (isoproterenol, especially), or stimulation of the sympathetic innervation, are characteristically high.
4) With pilocarpine stimulation, both calcium and amylase are significantly less than values obtained with isoproterenol stimulation.
5) With stimulation of the parasympathetic nerve, however, while amylase levels in the saliva are low throughout 90 minutes of continuous stimulation, calcium levels are virtually as high as those seen with isoproterenol stimulation.
6) The parallelism in levels of amylase and calcium in the gland tissue during secretion has been examined. It is concluded that secretion of the two need not occur in parallel.

## LIST OF REFERENCES


1. Alexander, R. L., Jr. Evaluation of an automatic calcium titrator. Clin. Chem. 17: 1171-1175, 1971.

2. Batzri, S., and Z. Selinger. Enzyme secretion mediated by the epinephrine β-receptor in rat parotid slices factor governing efficiency of the process. J. Biol. Chem. 248: 356-360, 1973.

3. Bazerque, P. M., C. J. Perec, and S. E. Terradas. Calcium in salivary gland of the rat after autonomic denervation. J. Dental Res. 51: 918-922, 1972.

4. Bogart, B. I. Fine structural localization of cholinesterase activity in the rat submandibular gland. J. Histochem. Cytochem. 18: 730-739, 1970.

5. Burgen, A. S. V. The secretion of potassium in saliva. J. Physiol. (London) 132: 20-39, 1956.

6. Castle, J. D., J. D. Jamieson, and G. E. Palade. Radioautographic analysis of the secretory process in parotid acinar cells of the rabbit. J. Cell Biol. 53: 290-311, 1972.

7. Dreisbach, R. H. Secretion of calcium by rat submandibular gland. Am. J. Physiol. 196: 645-648, 1959.

8. Dreisbach, R. H. Effect of pilocarpine on transfer of $Ca^{45}$ and $K^{42}$ to rat submaxillary gland and kidney. J. Pharmacol. Exptl. Therap. 131: 257-260, 1961.

9. Dreisbach, R. H. Calcium binding by rat submaxillary gland in vitro. Biochem. J. 82: 71-75, 1962.

10. Dreisbach, R. H. Regulation of $Ca^{45}$ transfer in rat salivary and lacrimal glands in vitro. Am. J. Physiol. 207: 1015-1020, 1964.

11. Dreisbach, R. H. Effect of isoproterenol on calcium metabolism in rat salivary gland. Proc. Soc. Exptl. Biol. Med. 116: 953-956, 1964.

12. Dreisbach, R. H. Effect of isoproterenol on calcium, protein, and electrolytes of rat submaxillary gland. Proc. Soc. Exptl. Biol. Med. 126: 279-281, 1967.

13. Dreisbach, R. H., and R. Taugner. Radioautography of $Ca^{45}$ in the rat salivary gland. Arch. Oral Biol. 14: 1395-1400, 1969.

14. Douglas, W. W., and A. M. Poisner. The influence of calcium on the secretory responses of the submaxillary gland to acetylcholine or to noradrenaline. J. Physiol. (London) 165: 528-541, 1963.

15. Gromet-Elhanen, Z., and T. Winnick. Microsomes as sites of α-amylase synthesis in the rat parotid gland. Biochim. Biophys. Acta 69: 85-96, 1963.

16. Hand, A. R. Nerve-acinar cell relationships in the rat parotid gland. J. Cell Biol. 47: 540-543, 1970.

17. Hand, A. R. Adrenergic and cholinergic nerve terminals in the rat parotid gland. Electron microscopic observation on permanganate-fixed glands. Anat. Rec. 173: 131-140, 1972.

18. Hebb, C. O., and G. W. Stavraky. The presence of glucose in the salivary secretion after administration of adrenalin. Quart. J. Exptl. Physiol. 26: 141-153, 1936.

19. Lacey, O. L. Statistical Method in Experimentation. 4th Edition. The Macmillan Company, New York, p. 101-131, 1960.

20. Mandel, I. D., R. Katz, A. Zengo, A. H. Kutscher, R. A. Greenberg, S. Katz, R. Scharf, and A. Pintoff. The effect of pharmacologic agents on salivary secretion and composition in man. I. Pilocarpine, atropine and anticholinesterases. J. Oral Therap. Pharmacol. 4: 192-199, 1967.

21. Marsters, R. W., T. D. Kinney, and K. Y. Lin. A micromethod for the determination of plasma amylase. Clin. Chem. 6: 130-139, 1960.

22. Martin, K., and A. S. V. Burgen. Change in the permeability of the salivary gland caused by stimulation and by catecholamine. J. Gen. Physiol. 46: 225-243, 1962.

23. Martinez, J. R., P. C. Adshead, D. O. Quissell, and G. J. Barbero. The chronically reserpinized gland as a possible model for cystic fibrosis. II. Composition and cilioinhibitory effect of submaxillary saliva. Pediat. Res. 9: 470-475, 1975.

24. Martinez, J. R., H. Holzgreve, and A. Frick. Micropuncture study of submaxillary glands of adult rat. Pflugers Arch. 290: 124-133, 1960.

25. Maurs, C., G. Herman, S. Busson, L. Ovtracht, and B. Rossignol. Regulation of protein secretion and metabolism in rat salivary gland. I. Induction of protein discharge by carbachol and norepinephrine in the rat parotid gland. Effect of atropine, propranolol and phentolamine. J. Microscopie 20: 187-196, 1974.

26. Monnard, P., and M. Schorderet. Cyclic adenosine 3',5'-monophosphate concentration in rabbit parotid slices following stimulation by secretagogues. Europ. J. Pharmacol. 23: 306-310, 1973.

27. Myers, V. C., A. H. Free, and E. E. Rosinski. Studies of animal diastases. VI. The determination of diastase (amylase) in blood. J. Biol. Chem. 154: 39-48, 1944.

28. Norberg, K. A., and L. Olson. Adrenergic innervation of the salivary glands in the rat. Z. Zellforsch. 68: 183-189, 1965.

29. Parks, H. F. On the fine structure of the parotid gland of mouse and rat. Am. J. Anat. 108: 303-329, 1961.

30. Rossignol, B., G. Herman, A. M. Chambaut, and G. Keryer. The calcium ionophore A23187 as a probe for studying the role of $Ca^{++}$ ions in the mediation of carbachol effects on rat salivary gland: Protein secretion and metabolism of phospholipid and glycogen. FEBS Letters 43: 241-246, 1974.

31. Rossignol, B., G. Herman, and G. Keryer. Inhibition by colchicine of carbamylcholine induced glycoprotein secretion by the submaxillary gland. A possible mechanism of cholinergic induced protein secretion. FEBS Letters 21: 189-194, 1972.

32. Schneyer, C. A. Salivary gland change after isoproterenol-induced enlargement. Am. J. Physiol. 203: 232-236, 1962.

33. Schneyer, C. A. Role of sympathetic pathway in secretory activity induced in rat parotid by feeding. Proc. Soc. Exptl. Biol. Med. 147: 314-317, 1974.

34. Schneyer, C. A., W. M. Galbraith, and L. B. Mellett. Unexpected β-adrenergic effects of the antitumor agent, cyclocytidine, on rat salivary glands. Proc. Soc. Exptl. Biol. Med. 148: 1206-1211, 1975.

35. Schneyer, C. A., and H. D. Hall. Comparison of rat salivas evoked by auriculotemporal and pilocarpine stimulation. Am. J. Physiol. 209: 484-488, 1965.

36. Schneyer, C. A., and H. D. Hall. Autonomic pathway involved in a sympathetic-like action of pilocarpine on saliva composition. Proc. Soc. Exptl. Biol. Med. 121: 96-100, 1966.

37. Schneyer, C. A., and L. H. Schneyer. Electrolyte level of rat salivary secretion. Proc. Soc. Exptl. Biol. Med. 101: 568-569, 1959.

38. Schneyer, C. A., and L. H. Schneyer. Electrolyte level of rat salivary secretion in relation to fluid flow rate. Am. J. Physiol. 199: 55-58, 1960.

39. Schneyer, L. H. Secretion of K and fluid by rat submaxillary during sympathetic nerve stimulation. Am. J. Physiol. 229: 1056-1061, 1975.

40. Schneyer, L. H., and N. Emmelin. Salivary secretion. In Gastrointestinal Physiology, ed. by E. D. Jacobson and L. L. Shanbour. MTP International Review of Science, Butterworths, London, p. 183-226, 1974.

41. Schneyer, L. H., and C. A. Schneyer. Regulation of salivary gland amylase activity. Ann. N. Y. Acad. Sci. 85: 189-200, 1960.

42. Schneyer, L. H., J. A. Young, and C. A. Schneyer. Salivary secretion of electrolytes. Physiol. Rev. 52: 720-777, 1972.

43. Schramm, M. Secretion of enzymes and other macromolecules. Ann. Rev. Biochem. 36: 307-320, 1967.

44. Schramm, M. Amylase secretion in rat parotid slices by apparent activation of endogenous catecholamine. Biochim. Biophys. Acta 165: 546-549, 1968.

45. Schramm, M., and A. Bdolah. The mechanism of enzyme secretion by the cell. III. Intermediate stages in amylase transport as revealed by pulse labelling of slices of parotid gland. Arch. Biochem. Biophys. 104: 67-72, 1964.

46. Schultz, G., J. G. Hardman, K. Schultz, G. E. Baird, and E. W. Sutherland. The importance of calcium ions for the regulation of guanosine 3',5' cyclic monophosphate levels. Proc. Natl. Acad. Sci., USA 70: 3889-3893, 1973.

47. Selinger, Z., and E. Naim. The effect of calcium on amylase secretion by rat parotid slices. Biochim. Biophys. Acta 203: 335-337, 1970.

48. Selinger, Z., E. Naim, and M. Lasser. ATP dependent calcium uptake by microsomal preparations from rat parotid and submaxillary gland. Biochim. Biophys. Acta 203: 326-334, 1970.

49. Snell, R. S. The histochemical appearances of cholinesterase in the parotid salivary gland of the rat. Z. Zellforsch. 49: 330-338, 1959.

50. Snell, R. S., and J. R. Garrett. The distribution of cholinesterase in the submaxillary and sublingual salivary glands of the rat. J. Histochem. Cytochem. 5: 236-245, 1957.

51. Taugner, R., and R. H. Dreisbach. In vitro cellular dynamics by radioautography: Calcium-45 in rat salivary gland. J. Pharmacol. Exptl. Therap. 180: 657-663, 1972.

52. Wallace, D., and M. Schramm. Calcium and the exportable protein in rat parotid gland parallel subcellular distribution and concomitant secretion. Europ. J. Biochem. 21: 433-437, 1971.

53. Yoshida, Y., R. L. Sprecher, C. A. Schneyer, and L. H. Schneyer. Role of β-receptors in sympathetic regulation of electrolytes in rat submaxillary saliva. Proc. Soc. Exptl. Biol. Med. 126: 912-916, 1967.

54. Young, J. A., and E. Schogel. Micropuncture investigation of sodium and potassium excretion in rat submaxillary saliva. Pflugers Arch. 291: 99-106, 1966.

GRADUATE SCHOOL
UNIVERSITY OF ALABAMA IN BIRMINGHAM
DISSERTATION APPROVAL FORM

Name of Candidate Choogiart Sucanthapree

Major Subject Physiology and Biophysics

Title of Dissertation Autonomic Regulation of Calcium and Amylase Secretion by Rat Salivary Glands

Dissertation Committee:

Leon H. Schneyer, Chairman — Charlotte A. Schneyer

Geraldine M. Emerson

Richard J. Krenzelok

Gerald L. Carlson

Director of Graduate Program Warren S. J. Rehm

Dean, UAB Graduate School L. B. Baker

Date 3 September 1976